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Editorial

Dear Readers,

We present to you the second issue of our journal for 2024. In this issue, we have published 1 original article, 2 review and 1 case reports and and that we think you will read with pleasure and interest. We hope that your scientific support will continue to increase in 2024. We would like to thank everyone who contributed to our journal for their support and contributions.

Best Regards.

Eurasian Journal of Toxicology Editorial Board

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Antituberculosis Drug-Induced Hepatotoxicity: Preclinical Benefit of Glutamine

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Abstract

Introduction: The use of rifampicin/isoniazid/pyrazinamide/ethambutol (RIPE) for the treatment of tuberculosis may cause hepatotoxicity. Glutamine (Gln) is an important amino acid with potential cell-regulatory and cytoprotective capabilities.

Objective: This study assessed the ability of Gln to prevent RIPE-induced hepatotoxicity in adult Wistar rats.

Materials and Methods: Thirty adult Wistar rats (both sexes) weighing 200-250 g were used. The rats were randomized into 6 groups of n=5/group and were orally administered with the experimental agents daily for 30 days as follows: Groups 1-3 were administered with ([Control] normal saline, 0.2mL), Gln (80mg/kg) and RIPE (Rifampicin 150, isoniazid/75, pyrazinamide 400 and ethambutol 275 mg/kg), respectively. Groups 4-6 were supplemented with Gln (20mg/kg, 40mg/kg and 80mg/kg) prior to the administration of RIPE, respectively. On day 31, the rats were weighed, anesthetized and blood samples were collected and assessed for biochemical markers. Liver samples were weighed and examined for histology and oxidative stress markers.

Results: RIPE significantly ($p<0.001$) decreased body weight, liver superoxide dismutase, glutathione peroxidase, catalase and glutathione levels when compared to the control. Liver weight, serum lactate dehydrogenase, gamma glutamyl transferase, aminotransferases, alkaline phosphatase, total bilirubin and liver malondialdehyde levels increased significantly ($p<0.001$) in RIPE -administered rats when compared to the control. RIPE caused hepatocelluar necrosis and steatosis in the liver of rats. However, the aforementioned RIPE-induced changes were mitigated in a dose-related fashion by Gln (20, 40 and 80 mg/kg) supplementation. Also, Gln supplementation restored liver histology.

Conclusions: Gln may be effective for the treatment of RIPE related hepatotoxicity.

Keywords: Antituberculosis drug, glutamine, liver, mitigation, toxicity

Introduction

The liver is a vital and primary organ involved in numerous functions including the detoxification and removal of waste products. [1] The metabolism of drugs takes place largely in the liver, which accounts for the organ's susceptibility to metabolism-dependent, drug-induced hepatotoxicity. Drug associated hepatotoxicity accounts for about one-half of the cases of acute liver failure and mimics all forms of acute and chronic liver diseases. [2 3] An estimated 1000 clinically used drugs have hepatotoxic potential. [4] The pathogenesis of drug-induced hepatotoxicity usually involves the participation of a drug and/or its metabolites, which affects cell biochemistry or stimulates an immune response. Each hepatotoxic drug is associated with a characteristic signature concerning the pattern of injury and latency. [5] However, some drugs may have more than one signature. Drug-induced hepatotoxicity could be characterised by unpredictable and idiosyncratic reactions, which may occur on a background of an increased rate of mild asymptomatic liver injury that may be difficult to recognize. [5]

Isoniazid/rifampicin/pyrazinamide/ethambutol (RIPE) is a frequently used antituberculosis drug combination. It has significantly reduced the health menace associated with TB infection in endemic regions .[6] However, RIPE is one of the known groups underlying idiosyncratic hepatotoxicity worldwide. [7] RIPE may cause hepatotoxicity in 5%–28% of tuberculosis (TB) patients on therapy. [8] Hepatotoxicity related to RIPE has been attributed to isoniazid [9] which can be aggravated by rifampicin. [10] Also, pyrazinamide intermediaries (pyrazinoic acid and 5- hydroxy pyrazinoic acid) can potentiate isoniazid associated hepatotoxicity. [11] Hepatotoxicity caused by RIPE is often accompanied by notable changes in serum liver markers [6] and hepatocellular changes such as necrosis, apoptosis and steatosis. [7] The precise mechanism of RIPE-induced hepatotoxicity is not clear, but may involve oxidative stress marked by lipid peroxidation and inflammation. [7]

L-glutamine (Gln) is an important amino acid that accounts for 60% of free amino acids in the body. [12] It has been associated with lots of vital physiological functions. It is a primary energy supply substance for mitochondria to produce adenosine triphosphate. [13]Gln plays essential functions in nitrogen and carbon skeleton exchange in different tissues, where it regulates many biochemical functions.[14] It has immunoregulatory and cell-regulatory

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capabilities, as reported in recent investigations. [15] Gln is essential for glutathione synthesis in tissues including the liver where it functions as a rate-limiter. [16] Glutathione is a potent ubiquitous antioxidant, which inhibits oxidative stress and is vital for drugs and endogenous substance metabolisms. [17, 18] In addition to the aforementioned activities, Gln has shown beneficial effects in diabetes, cancer, and neurodegenerative diseases. [13] It has been shown to reduce liver ischemia, reperfusion injury and alcohol-induced liver damage in experimental studies. [12] This study assessed the protective ability of Gln against RIPE-induced hepatotoxicity in Wistar rats.

Materials and Methods

Animals, drugs and experimental design

Animals: Adult Wistar rats (both sexes) weighing 200-250 g were procured from the animal house of the Department of Pharmacology, Faculty of Clinical Sciences, University of Port Harcourt, Rivers State, Nigeria. The rats were acclimated for 2 weeks under 12 h light: 12 h dark cycle at 25±2°C in the Department of Pharmacology/Toxicology, Faculty of Pharmacy, Niger Delta University, Nigeria where the study was performed.

Drugs/Chemicals: Isoniazid/rifampicin/pyrazinamide/ethambutol tables (RIPE) used were manufactured by Lupin Limited Chikalthana, Aurangabad India. Gln used was purchased from Qualikems Fine Chemical Private Limited, Gujarat, India. All other chemical compounds used were of analytical grades. RIPE (Rifampicin 150, isoniazid 75, pyrazinamide 400 and ethambutol 275 mg/kg) [19] and modified doses of Gln (20, 40, 80 mg/kg) [20] were used for the study.

Experimental design: Thirty adult Wistar rats (both sexes) weighing 200-250 g were randomized into 6 groups of n=5/group. The rats were orally administered with the experimental agents daily for 30 days as follows: Groups 1-3 were adminstered with the vehicle ([Control] normal saline, 0.2mL), Gln (80mg/kg) and RIPE (Rifampicin 150, isoniazid/75, pyrazinamide 400 and ethambutol 275 mg/kg), respectively. Groups 4-6 were supplemented with Gln (20mg/kg, 40mg/kg, 80mg/kg) prior to the administration of RIPE, respectively. On day 31, the rats were weighed and subjected to light diethyl ether anaesthesia and blood samples (5mLs) were collected in non-heparinized tubes. The blood samples were (centrifuged at 3000 rpm for 15 minutes) and sera were collected and assessed for biochemical markers. Subsequently, the rats were dissected, liver tissues were collected cleaned and weighed. Liver tissues were rinsed in cold saline and homogenized in phosphate buffer. The homogenates were centrifuged (3000 rpm for 15 minutes) and the supernatants were decanted and assayed for oxidative stress markers. Also, liver tissues were collected and fixed in 10% neutral buffered formalin for histological study.

Ethical consideration

This study was approved with the number NDU/PHARM/PCO/AEC/078 by the Research Ethics Committee of the Department of Pharmacology/Toxicology, Faculty of Pharmacy, Niger Delta University, Nigeria. The guide for the care and use of laboratory animals, 8th edition was used for the study.

Biochemical and histological evaluations

Biochemical markers: Serum lactate dehydrogenase (LDH), gamma glutamyl transferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total bilirubin (TB) were measured using standard laboratory reagents.

Liver oxidative stress maker assay: Malondialdehyde (MDA) was estimated using the procedure described by Buege and Aust, 1978. [21] Superoxide dismutase (SOD) was assayed as explained by Sun and Zigman, 1978. [22] Glutathione peroxidase (GPx) was assessed using the protocol described by Rotruck *et al*., 1973. [23] Catalase (CAT) was assayed as described by Aebi 1984. [24] Glutathione (GSH) was measured using the method reported by Sedlak and Lindsay 1968. [25]

Liver histology: The liver tissues were fixed in 10% neutral buffered formalin for 24h and dehydrated in ascending concentrations of ethyl alcohol solution. Liver tissues were processed and imbedded in paraffin block. Liver sections (3-4µm) were obtained from the paraffin blocks, mounted on slides and stained (hematoxylin-eosin). Stained sections were examined using a microscope (Nikon, Eclipse E200-LED, Tokyo, Japan).

Statistical analysis

Data as mean values with standard error of mean. Analysis of variance (ANOVA), complimented by Tukey's *post-hoc test* were used for data analysis with the aid of GraphPad Prism version 4.03 (GraphPad software Inc., San Diego, CA, USA). *P* values <0.05, <0.01 and <0.001 were considered significant.

Results

Effect of glutamine on body and liver weights of RIPE-administered rats

Administered Gln (80mg/kg) had no significant ($p>0.05$) effects on the body and liver weights whereas RIPE decreased body weight and increased liver weight significantly at $p<0.01$ when compared to the control **(Table 1).** But Gln supplementation significantly restored the body and liver weights at 20mg/kg ($p<0.05$), 40mg/kg ($p<0.01$) and 80 mg/kg ($p<0.01$) when compared to RIPE **(Table 1).**

Table 1: Effects of glutamine on the body and liver weights of RIPE-administered rats

Treatment (mg/kg)	FBW (g)	ALW(g)	RLW (%)
Control	290.9±20.2	6.56±0.54	2.26±0.12
Gln 80	297.6±20.4	6.37±0.32	2.14±0.27
RIPE	162.1±17.5*	10.01±0.45*	6.18±0.43*
Gln 20 + RIPE	200.3±19.4 [a]	8.21±0.32[a]	4.10±0.16 [a]
Gln 40 + RIPE	260.6±20.8 [b]	6.88±0.56 [b]	2.64±0.09[b]
Gln 80 + RIPE	271.5±17.8 [b]	6.56 ±0.98[b]	2.42±0.41[b]

Gln: Glutamine, RIPE: Rifampicin/isoniazid/pyrazinamide/ethambutol, FBW: Final body weight ALW: Absolute liver weight, RLW: Relative liver weight, Data as mean ± SEM, n=5, *p<0.01 Significant difference when compared to control, a p<0.05 and bp<0.01 Significant difference when compared to RIPE. SEM: Standard error of mean, ANOVA (Analysis of variance)

Effect of glutamine on serum biochemical markers of RIPE-administered rats

Serum AST, GGT, ALP, TB, ALT and LDH levels did not differ (p>0.05) from the control in Gln (80 mg/kg) administered rats, but were increased significantly (p<0.001) in RIPE- administered rats when compared to the control **(Table 2).** However, AST, GGT, ALP, TB, ALT and LDH levels were restored by Gln supplementation at 20 mg/kg (p<0.05), 40 mg/kg (p<0.01) and 80 mg/kg (p<0.001) when compared to RIPE **(Table 2).**

Effect of glutamine on liver oxidative stress markers of RIPE-administered rats

The administration of Gln (80 mg/kg) had no significant (p>0.05) effects on liver GPx, CAT, GSH, SOD and MDA levels when compared to the control. Administered RIPE decreased GPx, CAT, GSH, and SOD, but increased MDA levels significantly at p<0.001 when compared to the control (**Table 3).** Nonetheless, Gln supplementation restored liver GPx, CAT, GSH, SOD and MDA levels at 20mg/kg (p<0.05), 40mg/kg (p<0.01) and 80 mg/kg (p<0.001) when compared to RIPE **(Table 3).**

Effect of glutamine on liver histology of RIPE-administered rats

Normal liver histology was observed in the control rats **(Figure 1a),** but hepatocyte necrosis, inflammatory cell infiltrations **(Figure 1b)** and steatosis **(Figure 1c)** were noted in RIPE- administered rats. Steatosis **(Figure 1 d)** and inflammatory cells infiltration **(Figure 1e)** were observed in rats supplemented with Gln (20 mg/kg), and (40 mg/kg), respectively whereas normal liver histology **(Figure 1f)** was observed in rats supplemented with Gln (80 mg/kg).

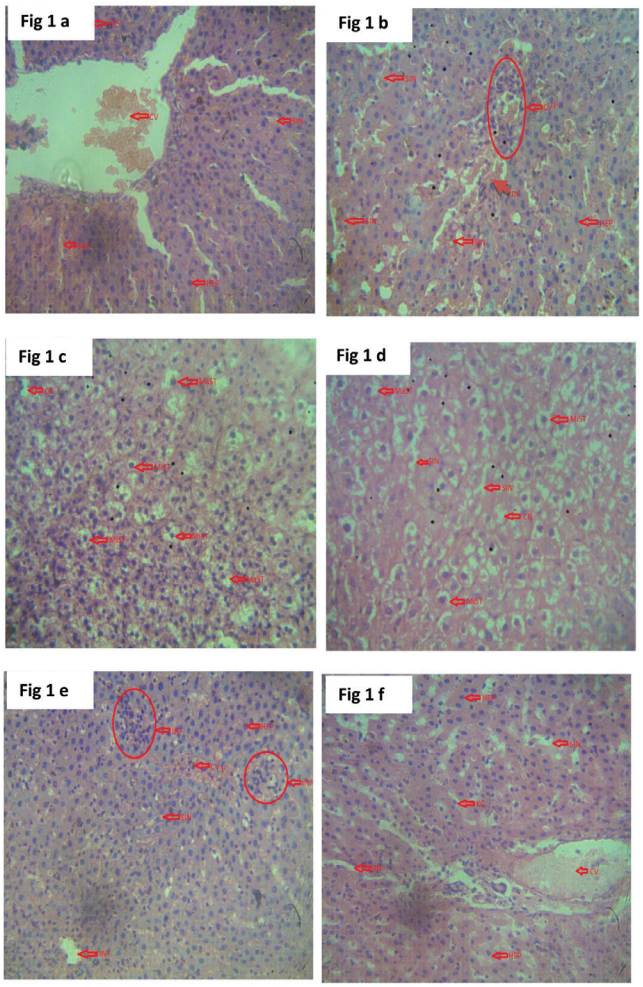

Figure 1 a-f: are liver micrographs. **Figure 1a:** Control, Figures b and c: RIPE administered rats, **Figures d-e:** Supplemented with glutamine (20 mg/kg), glutamine (40 mg/kg) and glutamine (80 mg/kg). **SIN:** Normal Sinusoids, **HEP:** Normal hepatocytes, **HN:** Hepatocyte necrosis, **CVF:** Central vein with inflammatory cells, **HV:** Hepatic vein, **CV:** Normal central vein. **INF:** Inflammatory cells, **MIST:** Microvesicular steatosis, **MAST:** Macrovesicular steatosis, **CB:** Councilman body, KC; Kuffer cells. X 400 (Hand E)

Table 2: Effect of glutamine on serum liver biomarkers of RIPE-administered rats

Treatment (mg/kg)	AST (U/L)	ALT(U/L)	ALP(U/L)	TB(g/dL)	LDH(U/L)	GGT(U/L)
Control	38.20±2.86	35.80±2.07	45.82±4.86	8.40±0.60	25.45±2.34	0.65±0.01
Gln 80	37.81±3.87	35.61±3.87	43.00±5.21	7.71±0.45	23.72±3.62	0.68±0.06
RIPE	141.02±12.7*	149.00±12.0*	184.20±13.7*	26.43±3.41*	110.66±18.1*	2.23±0.04*
Gln 20 + RIPE	95.22±9.12[a]	99.31±10.6[a]	123.21±17.8[a]	17.80±1.54[a]	70.05±7.21[a]	1.61±0.09[a]
Gln 40 + RIPE	71.20±8.13[b]	72.20±7.13[b]	80.00±6.35[b]	13.41±1.51[b]	48.10±5.72[b]	1.13±0.05[b]
Gln 80 + RIPE	44.12±4.09[c]	38.27±4.73[c]	56.20±6.99[c]	9.00±0.37[c]	27.52±3.54[c]	0.71±0.08[c]

Gln: Glutamine, RIPE: Rifampicin/isoniazid/pyrazinamide/ethambutol, AST: Aspartate aminotransferase, LDH: Lactate dehydrogenase, ALT: Alanine aminotransferase, GGT: Gamma-glutamyl transferase, ALP: Alkaline phosphatase, TB: Total bilirubin, n=5, Data as mean± SEM, *p<0.001 Significant difference when compared to control, ap<0.05, bp<0.01 and c p<0.001 Significant difference when compared to RIPE. SEM (Standard error of mean), ANOVA (Analysis of variance).

Table 3: Effect of glutamine on liver oxidative stress markers of RIPE-administered rats

Treatment (mg/kg)	CAT (µg/mg protein)	SOD (u/mg protein	GSH (u/mg protein)	GPx (u/mg protein)	MDA (nmol/mg protein)
Control	33.91 ± 3.05	25.42 ± 2.01	23.20± 3.83	35.41 ± 3.54	0.16 ± 0.07
Gln 80	34.21 ± 3.30	27.69 ± 3.12	23.41 ± 2.91	37.05 ± 2.93	0.15 ± 0.01
RIPE	12.60 ± 2.28*	7.01 ± 0.68*	5.60 ± 0.29*	13.61 ± 0.37*	0.98 ± 0.03*
Gln 20 +RIPE	16.81 ± 1.58a	11.20 ± 1.64a	8.41 ± 0.43a	18.86 ± 1.33a	0.58 ± 0.07a
Gln 40+ RIPE	22.80 ± 3.66b	15.31 ± 1.37b	12.60 ± 0.24b	25.01 ± 3.25b	0.32 ± 0.06b
Gln 80+ RIPE	30.89 ± 4.37c	23.22± 3.67c	20.98 ± 3.71c	32.21 ± 4.74c	0.19 ± 0.06c

Gln: Glutamine, RIPE: Rifampicin/isoniazid/pyrazinamide/ethambutol, CAT: Catalase, SOD: Superoxide dismutase, GSH: Glutathione, GPx: Glutathione peroxidase, MDA: Malondialdehyde, n=5, Data as mean ± SEM (Standard error of mean), *p<0.001 Significant difference when compared to control, a p<0.05, bp<0.01, and c p <0.001 Significant difference when compared to RIPE, ANOVA (Analysis of variance)

Discussion

Drug-induced hepatotoxicity is the leading cause of liver injury and acute liver failure in the world. [26] In TB therapy, RIPE associated hepatotoxicity is a significant adverse effect, which manifests with a broad signs of clinical features, from altered serum liver biochemistry to liver failure. [7] Gln, an essential amino acid used by several cell types, including hepatocytes showed cytoprotective activity in animal models. [27] The current research assessed the protective effect of Gln on RIPE-induced hepatotoxicity in adult rats. In toxicity studies, the measurements of organ and body weights are imperative. [28] In this study, RIPE notably decreased body weight and increased liver weight in the treated rats. This is consistent with the reports by Naji *et al.* [29] on altered organ weight in anti-tuberculosis drug administered rats. The decreased body weight might be due to decreased appetite whereas increased liver weight might be due to inflammation induced by RIPE. [29] However, Gln supplementation restored body and liver weights in dose-related manner. This might be due to increased appetite [30] and decreased liver inflammation. [27] Hepatotoxicity is recognized by abnormal liver biochemistry characterized by altered serum AST, ALT, ALP, GGT, TB and LDH with or without clinical symptoms. [31] In the RIPE-administered rats, this study observed elevated serum AST, ALT, ALP, GGT, TB and LDH levels. This is in agreement with the observations by Naji *et al.,* [29] who reported elevated levels of the aforementioned biochemical markers in rats administered with anti-tuberculosis drug. The elevated serum biochemical markers may be related to enhanced susceptibility and damage of the hepatocyte cell menbrane caused by RIPE leading to increased activities of serum biochemical markers. [29] But Gln supplementation, in a dose-related fashion restored serum biochemical markers. It can be suggested that Gln might have restored serum biochemical markers by maintaining liver plasma membrane integrity thus inhibiting the leakage of biochemical markers via the membrane. Recognizing the pattern of liver injury is vital. It helps establish a differential diagnosis and guide diagnostic evaluation. Assessing the pattern of liver injury is based on which liver enzyme elevation predominates. In this study, elevated ALT and AST levels were more prominent than ALP, which suggests hepatocellular injury. Similarly, Nagvi and others reported hepatocellular injury as the prominent pattern of liver injury associated with anti-tuberculosis drugs. [32]

Reactive oxygen species (ROS) which are by-product of normal metabolism have functions in cell signalling and homeostasis and are regulated by antioxidants. The regulation of cellular levels of ROS by antioxidants prevents their reactive nature from causing damage to key cellular components (DNA, protein, and lipids). But when the cellular antioxidant capacity is overwhelmed by ROS, oxidative stress occurs. Oxidative stress impacts have been linked with drug-induced toxicities including hepatotoxicity. [33] In this study, RIPE caused remarkable oxidative stress heralded by depleted liver antioxidants (GSH, CAT, GPx and SOD). The observation agrees with the findings by Sahu *et al.* [19] who reported depleted liver antioxidants in anti-tuberculosis drug administered rats. Studies have related depleted liver antioxidants to the metabolites of anti-tuberculosis drugs. Rifampicin induces CYP2E1, a member of the cytochrome P450 family, which facilitates the biotransformation of isoniazid to hydrazine its toxic metabolite. Hydrazine then reacts with the sulfhydryl content of GSH, depleting GSH and other antioxidants, thus exposing the liver to oxidative stress. [34] Rifampicin can increase isoniazid biotransformation to isonicotinic acid which is hepatotoxic. [35] Also, the intermediaries (pyrazinoic acid and 5- hydroxy pyrazinoic) of biotransformed pyrazinamide have been shown to cause oxidative stress. [11] Nonetheless, Gln supplementation restored liver antioxidant levels in a dose-related fashion. This may be related to the ability of Gln to inhibit the induction of oxidative by RIPE and/or its metabolites. Gln may have also increased liver antioxidant capacity especially GSH, because it is the rate-limiter for the production of GSH. [16]

Lipid peroxidation is a product of ROS action on polyunsaturated fatty acids. It is a chain phenomenon that begins with ROS giving an electron to a methylene

carbon in a polyunsaturated fatty acid, which reacts with molecular oxygen to form a peroxyl radical. LPO results in damage to membrane, inhibition of enzymes and cross-linking of protein-protein, which can cause cell death. [36] In this study, MDA, a primary LPO yardstick was elevated in the liver of RIPE administered rats. Similarly, Liu *et al.* [37] reported increased liver MDA level in anti-tuberculosis drug administered rats. Liu *et al.* [37] stated that this may be related to anti- tuberculosis drug-induced generation of ROS mediated by cytochrome P450 2E1 (CYP2E1) causing the oxidation of liver polyunsaturated fatty acids leading to LPO. However, Gln supplementation decreased LPO marked by restored liver MDA levels in a dose-related fashion. Gln might have inhibited the generation of ROS, thus preventing the oxidation of liver polyunsaturated fatty acids by RIPE.

Histology is an essential technique for evaluating the impact of test samples at tissue level. [38] In this study, histological study of the liver showed hepatocellular necrosis and steatosis in RIPE-administered rats. This is consistent with earlier liver morphological changes reported in anti-tuberculosis drug-administered rats by Saraswathy *et al.* 1998. [39] But various doses of Gln restored liver histology. Gln restored liver histology probably by inhibiting RIPE and /or metabolites from the induction of liver oxidative stress, thus preventing the damage of cellular contents (DNA, lipids and proteins).

Conclusion

Gln supplementation, in a dose-related fashion prevents RIPE-induced hepatotoxicity by restoring serum biochemical markers, liver oxidative stress markers and histology in rats.

Conflict of interest: None
Funding: None

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Review

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Botulism: Clinical Features, Laboratory Insights And Management Options

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Abstract

Botulism is a neuroparalytic disease caused by the neurotoxin produced by Clostridium botulinum (a gram-positive, anaerobic, endospore-forming bacillus). Botulinum neurotoxin (BoNT) is one of the most potent substances known, and seven toxin serotypes (serotypes A–G) have been identified. Type A serotype botulism is the most common cause of human botulism, and can occur in epidemic proportions. Botulism occurs after ingestion of food contaminated with BoNT, colonization of a wound by neurotoxin-producing Clostridium species, and exposure to botulinum neurotoxins by inhalation or injection. In all forms of botulism, progressive muscle weakness is usually seen, beginning in the cranial nerves and progressing in a proximal to distal manner to the extremities. This descending paralysis can lead to respiratory failure and death with involvement of the respiratory muscles. Treatment includes supportive care, intubation, and early administration of botulinum antitoxin.

Introduction

Botulism toxicity is caused by neurotoxins produced by *Clostridium botulinum* (*C. botulinum*) and occasionally by the closely related species *C. baratii* and *C. butyricum*[1]. *Clostridium botulinum* (*C. botulinum*) is a gram-positive, anaerobic, endospore-forming bacillus, and the causative agent is the botulinum neurotoxin (BoNT), one of the most potent biological substances known[2].

Clostridium botulinum is among the most resilient and efficient biological entities in nature and forms a spore that can withstand the harshest environmental conditions. In spore form, *C. botulinum* can be found ubiquitously in soil. It is estimated that spores can survive in liquid media for 30 years and under space conditions for many months[3]. When suitable environmental or laboratory conditions are provided (pH around 7.0, optimum growth at 35°C, and anaerobic conditions), *C. botulinum spores* can develop into toxin-producing bacilli[4]. *Clostridium botulinum* spores can withstand standard cooking and food processing measures, and for this reason, spores in foods preserved under appropriate conditions can germinate into the vegetative form and produce toxins. Consequently, the modern industrial canning technique was developed specifically to kill *C. botulinum* spores[3,4].

Botulism-related syndromes develop by exposure to botulinum neurotoxins through ingestion of preformed toxin (foodborne botulism), by colonization of a wound (wound botulism), or via the gastrointestinal tract (infant botulism and adult intestinal colonization botulism) by *Clostridium* species that produce neurotoxins, and via cosmetic or therapeutic injection (iatrogenic botulism)[1].

Epidemiology

Studies on botulism began in 1793 in Germany after an outbreak associated with blood sausages. *C. botulinum* was first isolated by Van Ermengem in 1897 from raw salted ham, which caused an epidemic that affected twenty-three people in Belgium and led to three deaths[5]. In 1949, Burgen and colleagues discovered that the botulinum neurotoxin inhibits neurotransmitter release. Later, a cellular mechanism of action was proposed that included three sequential steps, namely binding to neuronal acceptors, neuronal internalization, and intraneuronal action, and

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subsequently, the amino acid sequence of botulinum toxins was determined[6].

Outbreaks of foodborne botulism continue to be reported worldwide. The extent and duration of a botulism outbreak can vary depending on the type and source of exposure, as well as on whether the exposure is limited to a specific geographic location. The most common type of exposure to botulism is point source outbreaks, though intermittent common source outbreaks still account for a quarter of all outbreaks. Food preferences that vary by region indicate that the toxin type may also vary[7]. The use of biological agents to commit biocrime is a global problem. Due to its potential for use as a biological weapon, the storage and processing of BoNT requires enhanced safety measures and controls. BoNT is one of the most powerful and lethal biological agents known in nature, even with the lowest dose. In a person with an average body weight of 70 kg, 70 µg of ingested, 0.7-0.9 µg of inhaled, or 0.09-0.15 µg of intravenously administered concentrated BoNT/A toxin can be lethal[2]. It is not known whether exposure to purified toxin, such as might occur in the case of bioterrorism, would produce gastrointestinal signs and symptoms[1].

Pathophysiology

C. botulinum species are classified in four groups according to culture and serological characteristics[5]. Seven different botulinum toxin serotypes (BoNT A-G) have been identified through the study of botulism outbreaks in humans (BoNT/A, /B, /E and /F), birds (BoNT/C), cattle (BoNT/D), or isolated from soils (BoNT/G). The various BoNTs are produced by different *Clostridium botulinum* strains that exhibit heterogeneous bacteriological characteristics[8]. Most *Clostridium botulinum* strains produce a single antigenic type of toxin, but some strains produce two types of toxin, generally a large amount of one toxin and a small amount of the other (Table 1)[5]. BoNTs are single-chain polypeptides composed of a ≈50 kDa light chain and a ≈100 kDa heavy chain, linked by both disulfide bridges and non-covalent interactions functioning as a zinc-dependent endopeptidase[8]. Botulinum neurotoxin enters the bloodstream after ingestion, absorption from a colonized wound or intestine, inhalation, or injection. After entering the body, botulinum neurotoxins are transported by the circulatory system to the cholinergic synapses of the peripheral and cranial nervous system, where they affect the neuromuscular junctions. The toxin binds to high-affinity presynaptic receptors, and is then transported to the nerve cell by receptor-mediated endocytosis. The N-terminal heavy chain domain of BoNT facilitates the transport of the catalytic domain (light chain) from the endosomal membrane to the cytosol of the peripheral cholinergic nerve cell. After entering the cytosol, the light chain endoproteases prevent neurotransmitter (acetylcholine) release by specifically cleaving soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins in the postsynaptic nerve terminals. These proteins are the synaptosomal-associated protein 25 kDa (SNAP-25; cleaved by BoNT/A, BoNT/C, and BoNT/E), syntaxin (cleaved by BoNT/C), and synaptobrevin (also known as vesicle-associated membrane protein [VAMP], cleaved by BoNT/B, BoNT/D, BoNT/F, and BoNT/G)[2,3]. This causes descending symmetrical flaccid paralysis and cranial nerve palsy of varying severity[8]. In spite of their toxicity, they produce a prolonged but reversible effect at synapses[6,9].

In humans, botulism most often originates from strains producing toxin types A, B or E, and sometimes also from strains producing toxin type F[5]. Toxin type A in particular produces the most severe syndrome, and the proportion of patients requiring mechanical ventilation is high[9]. Toxin type B usually causes milder disease than type A and is the group with the lowest mortality. Cases of toxin types C, D, F and G are rare in humans, and this may be attributed to their lower presence in soil. Human cases caused by toxin type E are generally associated with the consumption of foods originating from water, producing a syndrome that includes gastrointestinal symptoms of varying severity[3]. Outbreaks involving toxin type E are the group with the shortest incubation period7. Moreover, although all toxin types can easily produce botulism in experimental animal models, conditions in the human intestine are not normally conducive to the germination of *C. botulinum* spores[9].

Clinical findings

Studies report that the symptoms of botulism are mostly food-related and most commonly develop due to toxin type

Table 1: Clostridium botulinum: Groups, Neurotoxins Formed, and Main Species Affected[2,5]

Group	Neurotoxin formed	Main species affected
Group I *Clostridium botulinum* (Proteolytic)	A	Humans, horses
	B	Humans, cattle, horses
	F	Humans
Group II *Clostridium botulinum* (Non-proteolytic)	B	Humans
	E	Humans, fish, birds
	F	Humans
Group III *Clostridium botulinum*	C	Birds, farmed chicken and pheasants, horses
	D	Cattle, sheep
Group IV *Clostridium argentinense*	G	No reported outbreaks, environmentally isolated
Group V *Clostridium baratii*	F	Humans
Group VI *Clostridium butyricum*	E	Humans

Table 2: Clinical criteria for early diagnosis of botulism[9,10]

1) Afebrile (≤37.8°C or ≤100.4°F)
2) At least one of the following symptoms: Blurred vision Double vision Difficulty speaking, including slurring Any change in voice, including hoarseness Dysphagia/accumulation of secretions/drooling Thick tongue
3) At least one of the following symptoms: Ptosis Extraocular palsy/decreased ability to track objects/tiredness caused by blocking light shining into the eyes Facial paresis/loss of facial expression/accumulation of secretions or milk/poor feeding/poor sucking when using a pacifier/tiredness while eating Fixed pupils Descending paralysis starting from the cranial nerves

A. Although the disease is most often associated with larger outbreaks, sporadic cases also exist. The reported incubation period ranges between 1-12 days, while the shortest reported incubation period is two hours. While the average time from the onset of the disease to hospitalization is two days, hospital admissions can vary up to a period of 14 days. The toxin dose exposed to is closely related to the incubation period and the severity of the disease[1].

The most common signs and symptoms of botulism are descending paralysis, dysphagia, weakness, diplopia, blurred vision, dysphonia, and dysarthria. Additionally, nausea, vomiting, ptosis, respiratory distress and respiratory failure may be observed in many patients[1,10]. There is a high need for mechanical ventilation in patients presenting with respiratory distress, especially in botulism due to toxin type A9. It is reported that most of these patients are admitted to hospital within the first 48 hours from the onset of the disease and are intubated1. Therefore, public health authorities need to take this situation into consideration, especially in cases where bioterrorism is suspected[4].

Less frequently, ocular palsy, dyspnea, dry mouth, abdominal pain, dizziness, abnormal pupillary reaction, and dilated pupils may also occur. Involvement of one or more cranial nerves may be seen accompanying the symptoms at the time of admission to hospital. These patients should be evaluated for weakness that progresses in a proximal to distal manner and for respiratory involvement[1,8]. Table 2 presents clinical criteria for early diagnosis of botulism[9,10].

The botulism patient is usually alert and has no fever provided there is no secondary infection. Fever is also rare in infants and young children, but may be more common than in adults10. When clouding of consciousness occurs in patients, respiratory failure, drug or alcohol use, pre-existing disease, or concurrent infection should be considered. Postural hypotension may occur[11].

Botulism causes prolonged flaccid paralysis which can last weeks or months. Aspiration of oral secretions and stomach contents may occur due to loss of protective airway reflexes. In the acute phase, death usually occurs due to early respiratory failure, while in the later stages of the disease, it is caused by long-term intensive care complications such as ventilator-associated pneumonia and deep vein thrombosis[9]. The mortality rate due to botulism increases with age. Mortality rates have decreased from 50% to less than 1% in recent years thanks to the developments in intensive care. Mortality rates are higher in wound botulism patients (15% to 17%) and lower in infant botulism patients (less than 1%). In recovered patients, it might not be possible for muscle strength and endurance to return to normal for up to 1 year, and long-term psychological problems may also occur[11].

Syndromes related to botulism

1. **Foodborne botulism:** Symptoms may appear within 12-36 hours or up to eight days after consuming food containing botulinum neurotoxin[5]. Foodborne botulism usually occurs due to inadequate storage conditions and exposure to undercooked home-canned foods or after consuming contaminated food from commercial sources[11]. The first symptoms may be nausea and vomiting. However, these symptoms may be caused by the toxin or may be due to other products of *C. botulinum* or other degradation products. Initially, the toxin causes symptoms such as double vision, inability to focus, ptosis, dry mouth, difficulty in speaking clearly (dysphonia), and dysphagia. Failure of the body muscles, especially the respiratory and cardiac muscles, can result in death. Some patients may be misdiagnosed with Guillain-Barré syndrome or myasthenia gravis instead of botulism[5]. Foodborne botulism outbreaks usually affect a small number of people. However, since large outbreaks are possible ("epidemic potential"), foodborne botulism is a public health emergency[9]. A botulism outbreak may escalate into a mass casualty incident when emergency medical services resources are inadequate in relation to the number and severity of cases. This can put great pressure on hospitals, as it requires long-term use of emergency services and frequently, intensive care facilities[12].
2. **Wound botulism (exposure to botulinum neurotoxin from a wound colonized by bacteria):** Wound botulism may develop in traumatic injuries and infected surgical wounds. The anaerobic conditions that occur in a wound abscess provide a suitable environment for bacteria to proliferate, and infection begins when BoNT passes into the bloodstream. Wound botulism is a risk for drug users who inject subcutaneously or intramuscularly and can cause outbreaks among drug users due to shared-use products. In some cases of wound botulism, nausea and vomiting may occur due to drug or alcohol use, botulism-induced ileus, diplopia, or coinfection (e.g., bacteremia, pneumonia)[1,3,5].

3. **Inhalation botulism:** This may occur in laboratory workers after inhalation of toxins and may develop after inhalation of spores in drugs[5]. It was first identified among German research laboratory workers in 1962. Clinical symptoms are similar to botulism caused by ingestion of the toxin, except for the absence of gastrointestinal symptoms (e.g., nausea, vomiting, abdominal cramps, diarrhea)[4].
4. **Iatrogenic botulism:** This may develop after exposure to botulinum neurotoxin through injection of high-concentration botulinum toxin for cosmetic or therapeutic purposes[13].
5. **Infant botulism:** This is seen in babies under one year old and has the highest incidence between 6 weeks and 6 months. Infant botulism occurs as a result of ingestion of spores that produce the toxin in vivo[11]. Following ingestion, the spores undergo germination and the organism can become established in the intestines of young infants. The most important reason for this is that the normal intestinal flora is not yet established enough to prevent this colonization. The infant experiences constipation, generalized weakness, and progressive paralysis and other neurological symptoms. Samples were taken from some patients diagnosed with sudden infant death syndrome and the presence of *C. botulinum* spores was detected[5]. Honey consumption is a risk factor for the disease, but this probably accounts for only 20% of cases. The clinical findings resemble adult forms of the disease, with common symptoms such as difficulty in sucking and swallowing, change in voice tone, ptosis, and drooping neck, and these symptoms may progress to general flaccid paralysis and respiratory failure[13].
6. **Adult intestinal colonization botulism:** This is associated with intestinal abnormality or surgery and/or antibiotic treatment. In most adults, the intestinal flora prevents the establishment of any ingested *C. botulinum* spores. Since it rarely occurs or is poorly recognized, few adult cases of this type of botulism have been reported[5,13].

Diagnostic methods for detecting botulism

We know that patients with suspected botulism receive a delayed diagnosis, as complete blood count and cerebrospinal fluid (CSF) examination and imaging methods are generally evaluated as normal. After 7 days of the disease, the amount of CSF protein may be found to have increased[14]. The Tensilon test (edrophonium test) can be used to rule out myasthenia gravis. Electrodiagnostic and neuromuscular conduction tests can be performed to reveal muscle weakness, and in the event of a large outbreak, electrodiagnostic studies can differentiate diseases such as Guillain-Barré syndrome and myasthenia gravis from botulism, thus contributing to the change of treatment. Repetitive nerve stimulation (RNS), electromyography (EMG), and nerve conduction studies (NCS) are recommended as electrodiagnostic studies. The distinctive classical findings seen in botulism include an increase in compound motor nerve action potential amplitude, repetitive nerve stimulation (RNS) rates of 30-50 Hz, fibrillation, decreased use of muscle units, decreased duration of muscle unit potentials in electromyography (EMG), and decreased motor-evoked amplitude on a nerve conduction study (NCS) when other findings are normal[15]. However, electrodiagnostic studies may be normal, and therefore unhelpful, in the early stages of the disease[15].

The diagnosis of symptomatic botulism is confirmed by identifying the neurotoxin:

1. Botulism neurotoxin in the stool, serum and gastric fluid,
2. Growth of *Clostridium* species in the stool or wound culture (those that produce neurotoxins),
3. The diagnosis is confirmed by detecting botulinum neurotoxin in food consumed by the symptomatic person[13.]

The gold standard for laboratory confirmation of botulism is the mouse bioassay[2,3]. Real-time polymerase chain reaction can detect *Clostridium* species growing in culture. The botulinum neurotoxin can be identified by distinguishing serotypes within hours using the mass spectrophotometry method. Sample collection, transportation and storage should be carried out in accordance with the laboratory's botulism recommendations[16,17].

Neurological observation of botulism

Patients initially experience nausea and vomiting, while cranial nerve palsy occurs in all patients over time. Respiratory failure and extremity weakness may occur due to involvement[18].

Respiratory observation of botulism

Around half of patients may require intubation due to involvement of the diaphragm or upper airway (preventing aspiration) muscles[1,18]. The need for intubation can be determined by spirometry, physical examination, respiratory rate, or nasal respiratory pressure measurement. Assessment with blood gas may not be reliable in the early period[19-20-21].

Treatment

Treatment includes supportive care, intubation and antitoxin administration. If botulism is suspected, it should be treated with botulinum antitoxin (BAT). If neurological findings continue to worsen after administering one vial of BAT, diagnoses other than botulism should be considered. BAT administration (ideally within the first 24 hours, preferably within 48 hours) leads to a decrease in mortality and morbidity. In BAT administration, skin sensitivity testing is

not required[22]. The antitoxin does not reverse paralysis, but halts the progression. BAT treatment may be reconsidered in children who continue to deteriorate neurologically despite dose-adjusted BAT treatment for foodborne botulism[23]. If botulism is suspected in pregnant women, BAT treatment should be performed[24]. Until she receives BAT, the mother should cease breastfeeding, and express and discard her milk in consultation with a breastfeeding specialist. Aminoglycosides, magnesium, clindamycin, tetracycline or calcium should be given to botulism patients only after careful assessment and appropriate monitoring[25,26]. Activated charcoal administered orally may cause morbidity in cases of ileus or aspiration[27,28]. The effectiveness of activated charcoal and plasmapheresis has not yet been fully proven[29]. Clinicians should be prepared for dry eye, ileus, pressure sores, urinary retention, and deep vein thrombosis[30-31]. Since toxin does not pass through the blood-brain barrier in botulism patients, it should be remembered that their cognitive functions are intact and psychological procedures should be applied[32,33].

Conclusion

- In crisis and emergency situations, early clinical diagnosis of botulism should be made by criteria.
- Botulism patients should be examined frequently.
- The airway should be protected.
- It should not be forgotten that the patients' cognitive functions are intact.
- BAT treatment should be given in case of doubt (regardless of the route of exposure).
- One should be prepared for an epidemic situation.

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Review

Eurasian Journal of Toxicology

An Overview Of New Oral Anticoagulant Toxicity

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Abstract

Atherosclerosis and thrombosis are the underlying causes of stroke and myocardial infarction. Anticoagulants have a significant role in both the prevention and treatment of these conditions. The difficulty of monitoring warfarin derivatives, which have been in use until recently, and their narrow therapeutic range paved the way for the development and use of new oral anticoagulants. Rivaroxaban, Apixaban, Edoxaban, and Betrixaban are Factor Xa inhibitors, while Dabigatran is a Thrombin (FIIa) inhibitor, and together they comprise the new oral anticoagulants. It is crucial to manage any accidental or intentional overdose with these new oral anticoagulants. The present review focuses on the characteristics of novel anticoagulants and their toxicological management.

Introduction

In the 20th and 21st centuries, the improvement in education and income levels and technological advancements contributed to the rise in average life expectancy. However, these developments have also had negative effects on human health. The increase in the means of transportation, the decline in physically demanding jobs, the rise in the workload of desk jobs, and technology dependence have resulted in a more sedentary lifestyle. The deterioration in dietary habits during this period, combined with a lack of physical activity, has contributed to the rise in obesity. Additionally, the increase in tobacco use over the past century played a role in the growing health problems. While all these factors contributed to the rise in the incidence of cardiovascular diseases, acute myocardial infarction and stroke have been among the leading causes of death. Anticoagulants play a vital role in preventing and treating these conditions. Indeed, Warfarin is effective in preventing thromboembolic events. However, its narrow therapeutic range, potential interactions with food and other medications, the need for INR monitoring, and individual variations in treatment response led scientists to explore alternatives that eliminate the need for laboratory monitoring while offering effective treatment with fixed doses.[1]

The Mechanism of Coagulation

Physiological coagulation occurring outside the blood vessels is called haemostasis, while pathological coagulation that occurs within the blood vessels is referred to as thrombosis. If the clot formed during coagulation is attached to the wall of a blood vessel, it is called a thrombus. When it moves freely through the bloodstream, it is called an embolus. Physiological or pathological coagulation occurs as a result of a systemic and complex interaction among platelets, endothelial cells, and coagulation factors. Arterial thrombi can lead to acute myocardial infarction, stroke, and gangrene of the proximal or distal extremities. Venous thrombi, on the other hand, can cause pulmonary embolism and phlebitic syndromes. The coagulation cascade consists of three phases: the platelet phase, the vascular phase, and the plasma phase. In the platelet phase, collagen exposed from the damaged vessel wall binds to the von Willebrand Factor (vWF) present in the plasma. As a result of this

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bridge formation, platelet adhesion takes place, and platelets are activated. Following adhesion, activated platelets release factors such as adenosine diphosphate (ADP), platelet-derived growth factor (PDGF), and vWF. At this point, ADP promotes the activation of additional platelets while also activating GPIIb/IIIa receptors. Fibrinogen promotes platelet aggregation via these receptors. When endothelial damage occurs, the release of prostaglandin I2 (PGI2, endothelial-derived) decreases, while the release of thromboxane A2 (TxA2, platelet-derived) increases. When endothelial damage is more significant, platelet aggregation and the release of FIII from the damaged endothelium trigger plasma coagulation factors, leading to the formation of FIIa (thrombin). Subsequently, thrombin converts fibrinogen into fibrin, forming a stable clot structure. In this mechanism, thrombin is the strongest platelet activator. There are three groups of drugs used in the treatment of conditions caused by thrombus formation. The first group is antithrombotic agents. This group consists of aspirin, a cyclooxygenase inhibitor; dipyridamole and cilostazol which are phosphodiesterase inhibitors; Clopidogrel, Ticlopidine, Ticagrelor, Cangrelor, and Prasugrel which are inhibitors of ADP receptor; and Abciximab, Eptifibatide, and Tirofiban which are glycoprotein (GP) IIb/IIIa inhibitors. The second group consists of anticoagulant agents. This group includes Warfarin and Dicumarol, which are vitamin K antagonists; Heparin, an inhibitor of thrombin (FIIa) and Factor Xa, as well as thrombin inhibitors, and Factor Xa inhibitors. The third group, namely the fibrinolytic group, includes drugs like Alteplase and Streptokinase, which are plasminogen activators.[2]

New Oral Anticoagulants (NOACs)

Anticoagulants are used in patients with atrial fibrillation (AF) for the prevention of stroke, in the prophylaxis and treatment of deep vein thrombosis (DVT), for the treatment of pulmonary embolism, as well as for secondary prevention of cardiovascular events. Standard heparin, which produces its anticoagulant effect by inhibiting Factor Xa and thrombin, has been in use for the past 50 years. Low molecular weight heparin (LMWH) was introduced for clinical use in the early 1990s. Another oral anticoagulant, the vitamin K antagonist Warfarin, has also been in use. The effectiveness of these drugs (warfarin) has been established, but their narrow therapeutic range, high potential for food and drug interactions, and the need for laboratory monitoring create challenges not only for patients but also for clinicians. As a result of scientific research to develop drugs that would not require monitoring, provide effective treatment with fixed doses, and have a low side effect profile, new oral anticoagulants (NOACs) were developed. Rapid onset of action, oral administration, minimal drug and food interactions, no need for laboratory monitoring, high effectiveness, and low side effect profile are the advantages of using new oral anticoagulants. On the other hand, they have some drawbacks, such as loss of efficacy if doses are missed, limited use in patients with impaired renal function, non-availability of monitoring methods, and absence of established dosing regimens to be followed in the presence of comorbidities. Looking at these two groups of anticoagulants from another perspective, a key difference noted is that the anticoagulants in the warfarin group have a delayed onset of action and require bridge therapy. NOACs are more expensive than the anticoagulants in the warfarin group. New oral anticoagulants include Fondaparinux, which is administered parenterally, Dabigatran, Apixaban, Betrixaban, Edoxaban, and Rivaroxaban, which are taken orally.3 Among these anticoagulants, Betrixaban has the longest duration of action.

Dabigatran

It is a direct thrombin inhibitor, also known as an FIIa inhibitor. It is used for stroke prophylaxis at a dose of 2x150 mg in patients with AF. It is used at a dose of 2x110 mg in patients at high risk of bleeding (a HAS-BLED score of 3 or higher). It is contraindicated in patients with a creatinine clearance of 30 ml/min or lower. Its average half-life is 14 to 17 hours. The most effective test for monitoring toxicity is the Thrombin Time (TT). Approximately 80% of dabigatran is excreted by the kidneys. Dabigatran is the only new oral anticoagulant that can be mostly removed through hemodialysis.4 In the event of Dabigatran toxicity, if the drug was taken within the last 2 to 4 hours, gastric lavage should be performed first, followed by the oral administration of 50 g or 1 g/kg of activated charcoal. Since at least 80% of dabigatran does not bind to serum proteins, hemodialysis should be considered, especially in patients with impaired renal function or in those whose renal function is progressively deteriorating.5 Additionally, idarucizumab, which has an affinity for dabigatran that is 350 times stronger than its affinity for thrombin, has been used in the treatment of dabigatran toxicity.6 Five grams of idarucizumab, administered in two equal doses, completely (100%) reversed the anticoagulant effect of dabigatran within 4 hours. The administration method should involve two equal infusions, each lasting 5 to 10 minutes, with a 15-minute interval between the infusions. The dilute thrombin time and ecarin clotting time (ECT) tests confirmed this result. If idarucizumab is unavailable, prothrombin complex concentrate (PCC) (COFACT) or activated PCC (FEIBA) can be administered at a dose of 50 U/kg, with the maximum being 4000 U. If the active bleeding site can be controlled, pressure or other methods should be used to manage the bleeding. Besides specific treatments, fluid replacement can be also used to maintain hemodynamic stability.

FactorXa Inhibitors

Rivaroxaban, Apixaban, Edoxaban, and Betrixaban are the drugs in this class. Rivaroxaban reaches the peak plasma concentration 3 hours after oral administration. Its half-life is 4 to 9 hours. The drug has minimal food and drug interactions. Its oral bioavailability is over 80%. Two-thirds of the drug is excreted via the liver, and one-third of the drug is eliminated as unchanged drug. The standard dose is 1x20 mg. The dose should be reduced to 1x15 mg in patients with a creatinine clearance of 15 to 49 mg/min. Apixaban does not cause organ toxicity or elevated liver enzymes in liver function tests. It has no food interactions. Its oral bioavailability is above average. Its half-life is approximately 12 hours. Apixaban is used at a dose of 2x5 mg. However, if the patient weighs less than 60 kg, is over 80 years old, or has a serum creatinine level above 1.5, and two of these conditions apply, the dose should be reduced to 2x2.5 mg. The half-life of Edoxaban is 9 to 11 hours. The standard dose of Edoxaban is 1x60 mg. However, if the patient weighs less than 60 kg, has a creatinine clearance of 15 to 49 mL/min, or takes a P glycoprotein inhibitor concurrently, the dose is reduced to 1x30 mg. Betrixaban is the newest oral FXa inhibitor, with the longest half-life, ranging from 19 to 27 hours. It is indicated for long-term thromboembolism prophylaxis, but it is not indicated for stroke prophylaxis in patients with AF.7 Additionally, NOACs are not recommended during pregnancy, in cases of advanced liver failure, severe kidney failure, or dialysis, and for patients with mechanical heart valves, those with moderate to severe mitral valve stenosis, or in individuals with antiphospholipid syndrome. Generally, the plasma levels of NOACs increase when the age of the patient is over 80 years when the patient weighs less than 60 kg, and in cases of renal failure. On the other hand, the efficacy of the anticoagulants may decrease in the obese population due to the reduction in plasma levels. Andexanet alfa has been reported to exhibit a rapid onset of action (within 2 to 5 minutes) by correcting thrombin formation and normalizing coagulation in patients, who were treated with rivaroxaban, apixaban, and edoxaban.8 The drug reduces anti-factor Xa activity by 79% with an initial bolus followed by a 2-hour infusion. Andexanet (recombinant Factor Xa) was approved by the FDA in 2018 and is effective in reversing the effects of FXa inhibitors. Andexanet does not require any dose adjustments for renal failure. It is believed that with the introduction of these antidotes, managing bleeding complications in patients treated with NOACs will become easier.9 In cases of Factor Xa toxicity, another option to be considered is 4F-PCC at a dose of 50 U/kg.10 If it is not available, the third option should be aPCC at a dose of 50 U/kg. If the drug was taken within 2 to 4 hours, gastric lavage followed by the administration of 50 g of activated charcoal should be carried out.11 The anti-Factor Xa chromogenic assay is the preferred method for assessing the anticoagulant activity of apixaban, edoxaban, and rivaroxaban.12

Table 1: Anticoagulants

Mechanism of anticoagulation	Parenteral	Oral
Trombin(FIIa), Fxa inhibitors	Heparin, Danaparoid	
Trombin inhibitors	Hirudin, Bivalurudin, Argatroban	Dabigatran
Fxa inhibitors	Fondaparinux	Rivaroxaban, Apixaban Edoxaban, Betrixaban
Vitamine K antagonist		Warfarine,Dikumarol

Table 2: Treatment of Toxication

Drug	Treatment of Toxication
Dabigatran (Hemodialysis suitable)	Gastric Lavage,Hemodialysis,Actived charcoal,İdaricuzimab(S) *
Apixaban (Hemodialysis non-suitable)	Gastric Lavage,Actived charcoal,Andexanet alfa(S) *, 4F-PCC**
Edoksaban (Hemodialysis non-suitable)	Gastric Lavage,Actived charcoal, Andexanet alfa(S) *, 4F-PCC**
Betrixaban (Hemodialysis non-suitable)	Gastric Lavage,Actived charcoal, Andexanet alfa(S) *, 4F-PCC**
Rivaroksaban (Hemodialysis non-suitable)	Gastric Lavage,Actived charcoal, Andexanet alfa(S) *, 4F-PCC**

*S:Specific, **4F-PCC:Four-Factor prothrombin complex concentrate

General Approach and Conclusion

Bleeding associated with hemodynamic instability, occurring in anatomically critical areas, requiring the transfusion of 2 or more units of red blood cells, or causing a 2 g/dL or higher drop in hemoglobin levels (if baseline values are known) should be considered major bleeding. When we say “critical bleeding areas”, we refer to intracranial hemorrhages (such as subdural, epidural, intraparenchymal, and subarachnoid),

Table 3: Dosage of Antidotes

Drug	Dosage of Antidote
Dabigatran	İdaricuzimab → 2*2,5 gr(5gr)
Apixaban	Andexanet alfa(S) * → 30mg/min totaly 400 mg IV bolus,after 4mg/min 2 hour infusion 4F-PCC** → 50U/kg
Edoksaban	Andexanet alfa(S) * → 30mg/min totaly 400 mg IV bolus,after 4mg/min 2 hour infusion 4F-PCC** → 50U/kg
Betrixaban	Andexanet alfa(S) * → 30mg/min totaly 400 mg IV bolus,after 4mg/min 2 hour infusion 4F-PCC** → 50U/kg
Rivaroksaban	Andexanet alfa(S) * → 30mg/min totaly 400 mg IV bolus,after 4mg/min 2 hour infusion 4F-PCC** → 50U/kg

*S:Specific, **4F-PCC:Four-Factor prothrombin complex concentrate

pericardial tamponade, hemothorax, intra- and retroperitoneal bleeding, bleeding in the respiratory tract, and bleeding in joints. In patients with ongoing bleeding and/or hemodynamic instability, local measures to control bleeding should be combined with fluid resuscitation.13 Intravenous use of isotonic crystalloids such as 0.9% NaCl or Ringer's lactate is recommended for fluid resuscitation. Hypothermia and acidosis should be corrected because these conditions can worsen coagulopathy and contribute to bleeding. In the event of bleeding occurring in the critical anatomical regions, it is crucial to consult the relevant medical unit. In the presence of symptomatic anemia and bleeding, erythrocyte suspension should be administered to maintain the hemoglobin level at 7 g/dL, or 8 g/dL if there is concomitant coronary artery disease. For patients who require three or more units of erythrocyte transfusion within one hour, activation of a massive transfusion protocol should be considered.14 For ES: FFP: PLT, the ≤ 2:1:1 formula is the most frequently applied. In trauma patients, early administration of tranexamic acid within the first 3 hours of admission is associated with reduced bleeding and lower mortality and should be considered for use.15 Tranexamic acid should be initiated with a bolus dose of 10 to 20 mg/kg, followed by repeated doses of 10 mg/kg every 8 hours. In individuals with liver disease, INR, PT, and aPTT may not be reliable indicators for assessing hemostatic function. For this reason, further evaluation and hematology consultation should be considered in case of doubt. When necessary and if available, cryoprecipitate transfusion can be administered to maintain fibrinogen levels >100 mg/dL. Reversing the effect of NOACs is not recommended unless there is major bleeding.

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Review

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Auditory Hallucination Associated With Metronidazole: A Case Report

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Abstract

This article presents a case of auditory hallucinations occurring in a mentally healthy young patient, which are associated with the administration of metronidazole. A 43-year-old female patient presented to the emergency department with complaints of auditory hallucinations, altered consciousness, and nausea after the use of metronidazole. During the physical examination, the patient was alert, oriented, and cooperative; cranial nerve examination, sensory and motor functions, and cerebellar tests were all normal; no signs of meningeal irritation were detected. This case underscores the importance of being vigilant regarding the potential neurological side effects associated with metronidazole use.

Keywords: Metronidazole; Auditory hallucinations; Side effects

Introduction

Metronidazole is an antibiotic belonging to the 5-nitroimidazole class, utilized in the treatment of a range of bacterial and protozoal infections. Due to the rarity of serious side effects, it has been safely used for decades. Common side effects associated with metronidazole include nausea, headaches, alcohol intolerance, a metallic taste in the mouth, and gastrointestinal discomfort[1-3]. Rare side effects include neutropenia, hemolytic-uremic syndrome, Stevens-Johnson syndrome, psychotic episodes, and encephalopathy[2,3]. Although neurological side effects are infrequently observed, early clinical identification and intervention are essential for optimal patient management[4-7]. This article presents a case of auditory hallucinations occurring in a mentally healthy young patient, which are associated with the administration of metronidazole.

Case Report

A 43-year-old female patient presented to the emergency department with complaints of auditory hallucinations, altered consciousness, and nausea after the use of metronidazole. It was learned that she had undergone a right hemicolectomy 8 days ago due to a transverse colon tumor. The patient, who had been undergoing inpatient treatment in the general surgery unit, was discharged on the seventh postoperative day with a prescription that included ciprofloxacin at a dosage of 750 mg administered twice daily and metronidazole at a dosage of 500 mg administered three times daily. The patient has been using ciprofloxacin for urinary tract infections over many years and has not experienced any side effects. After being discharged from the hospital, the patient reported confusion and auditory hallucinations that began approximately 1.5 hours after taking the metronidazole. She indicated that these symptoms persisted intermittently for a period of 6 hours. In the emergency department, the vital signs were stable (temperature: 36.4 °C, pulse: 94 bpm, O_2 saturation: 95%, respiratory rate: 20 breaths per minute, blood pressure: 164/76 mmHg). During the physical examination, the patient was alert, oriented, and cooperative; cranial nerve examination, sensory and motor functions, and cerebellar tests were all normal; no signs of meningeal irritation were detected. The patient's surgical site examination revealed

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no abnormalities. Laboratory analyses indicated elevated levels of C-reactive protein (CRP), leukocytes, platelets, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT), alongside decreased hemoglobin and hematocrit levels. As shown in Table 1. All laboratory values examined in the patient are included in Table 1. Furthermore, cranial diffusion-weighted magnetic resonance imaging (MRI) did not demonstrate any acute pathological findings. Consultations were conducted with both the neurology and general surgery departments. It was concluded that the patient's current symptoms could be associated with the administration of metronidazole. Following the discontinuation of metronidazole therapy, there was a notable improvement in the patient's symptoms. Upon follow-up five days later, the patient reported a complete resolution of her symptoms and stated that she had not experienced any further episodes of hallucinations.

Discussion

The improvement of auditory hallucinations after stopping metronidazole in our patient, who has no personal or family history of psychiatric disorders, indicates a possible causal link between metronidazole and the occurrence of auditory hallucinations. Metronidazole is an antibiotic that has been associated with various central nervous system adverse effects, including a condition referred to as "metronidazole-induced encephalopathy." The existing literature has primarily documented peripheral neuropathy as one of the neurological side effects linked to metronidazole8,9. Early diagnosis is essential for achieving a good prognosis in serious conditions such as encephalopathy. Most patients demonstrate significant improvement following the discontinuation of the offending medication.

Table 1: Laboratory results.

Laboratory Data	Value
CRP	43.8 mg/L
Leukocytes	10.83x10^3/uL
Hemoglobin	10.8 g/dL
Hematocrit	33.7%
Platelets	513.10^3/uL
Glucose	97 mg/dL
eGFR	109 ml/min/1.73m^2
BUN	6 mg/dL
Urea	13 mg/dL
Potassium	3.57 mmol/L
Sodium	143 mmol/L
Calcium	9 mg/dL
Creatinine	0.64 mg/dL
ALT	268 U/L
AST	149 U/L
ALP	143 U/L
GGT	147 U/L
Total Bilirubine	0.51 mg/dL
Direct Bilirubine	0.16 mg/dL
Amilase	72 U/L
Lipase	38 U/L

CRP: C-reaktif protein, GFR: Glomerüler Filtrasyon Hızı
ALT: Alanin aminotransferaz, AST: Aspartat Aminotransferaz
ALP: Alkalen Fosfataz, GGT: Gama Glutamil Transferaz

Conclusion

This case underscores the importance of being vigilant regarding the potential neurological side effects associated with metronidazole use.

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