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Is intralipid fat emulsion a promising therapeutic strategy on neurotoxicity induced by malathion in rats?

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Abstract. – AIM: Malathion is one of the most widely used organophosphate pesticides and herbicides. It has given rise to major clinical problems by its poisoning in all over the world. Malathion also a highly lipophilic agent, and tends to accumulate within lipid-rich tissue like a brain in the body, causing toxicity. Therefore, the study was aimed to investigate if there is a possible beneficial effect of using intralipid fat emulsion (IFE) on the neurotoxicity, and to detect it time-dependently at the beginning, 6th and 12th hours of M intoxication.

MATERIALS AND METHODS: Forty-eight rats were randomly divided into six groups including: control (C), Lipid (L) group (18.6 mL/kg oral IFE), Malathion (M) group (10 mg/kg oral M), M0L group (IFE treated after immediate from M), M6L group (IFE treated after 6 hours from M), M12L group (IFE treated after 12 hours from M).

RESULTS: M group in comparison with all others group, there was an increase in the total oxidant status (TOS) level. M group in comparison with C, L, M0L groups, it was seen significantly decrease in the total antioxidant capacity (TAC) level. Interestingly, M group in comparison with M6L and M12L groups, there was no significant difference among these groups in terms of the TAC levels. Although there was no significant difference among C, L and M0L groups in terms of both TAC and TOS levels, but was significant difference C, L groups in comparison with M6L, M12L groups in terms of TAC levels. C group in comparison with L, M0L, M6L, M12L groups in terms of TOS levels, there was no significant difference. These findings have indicated that IFE seriously reduced TOS levels in all the groups depending on time. Also, M0L group in comparison with M6L and M12L groups, there was significantly increase of the TAC levels. There was no statistically significant difference between M6L and M12L groups. These biochemical results were confirmed with immunohistochemical results.

CONCLUSIONS: The study has had some certain evidence that IFE is a promising safe therapy for acutely intoxicated cases by organophosphate. It is much more effective if used at the beginning of organophosphate poisoning. As such, there is no need to avoid using IFE in clinical practice.

Key Words:
Malathion, Intralipid fat emulsion, Neurotoxicity, Organophosphate intoxication, Rat.

Introduction

Malathion Toxicity

Organophosphorus pesticide poisoning is a major clinical problem in all over the world. An estimated 300,000 people died of organophosphate (OP) poisoning every year, and OP's are responsible for approximately two-thirds of these deaths that corresponds to a total of 200,000 per year[1]. Malathion (M) inhibits acetylcholinesterase and pseudocholinesterase, resulting in accumulation of acetylcholine and overstimulation of acetylcholine receptors in synapses of the nervous system, and neuromuscular junctions. There is a plenty of literature on neurotoxicological outcomes of acute exposure to M in laboratory animals. In many studies, neurotoxic effects such as various neuromotor, cholinergic, physiological, affective and cognitive disorders were reported at doses producing cholinesterase inhibition[2]. Also, acute exposure to M will cause body-wide symptoms, and their intensity will be dependent on the severity of exposure. Possible symptoms include nausea, diarrhea, cramps, excessive sweating, seizures and even death[3]. M is one of the most widely used OP pesticides due to its high selectivity to pests and low toxicity to human. Therefore, with the consideration as a prototype of OP's, M was selected as a toxic substance to create toxicity in the present study.

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Currently, it is focused on reversing the resultant cholinergic excessive effects through the use of atropine in the treatment of OP poisoning. Adjunctive treatments for suppressing OP-induced neurotoxicity include the usage of benzodiazepines and oximes. Medical therapy alone is not generally sufficient for severe OP poisoning. Because patients often require mechanical ventilation following the primary resuscitation[4].

Intralipid Fat Emulsion as a Therapeutic Agent

Intralipid is a brand name for the first safe fat emulsion for human use since approved in 1962. It is used as one of the major component in parenteral nutrition for patients who are unable to have sufficient nutrition via oral diet. It is an emulsion of soybean oil, egg phospholipids and glycerin, and available in a 10%, 20% and 30% concentration. Intralipid provides essential fatty acids such as linoleic acid, omega-6 fatty acid, alpha-linolenic acid (ALA) and omega-3 fatty acid. Recently, it has been used to resuscitate lethal local anesthetic drug toxicity[5]. Moreover, several animal studies supported that the use of intralipid as an antidote could treat bupivacaine toxicity[6]. But the exact mechanisms of antidotal action are not clear yet. Only "lipid sink" mechanism gives a potential explanation[7]. Intralipids probably move the fat-soluble drugs away from the site of toxicity and, thereby, may alleviate toxic effects of the fat-soluble drugs.

Hypothesis

The present study was planned with a hypothesis that Organophosphorus pesticides are highly lipophilic agents, and tends to be sequestrated into lipid-rich tissues like brain, causing neurotoxicity. If M is followed by intralipid fat emulsion (IFE) through the digestive system, its toxic effects are expected to minimize by the reduction of its absorption and plasma levels. To find out the best therapeutic gap for M poisoning, toxic substance was applied at the different intervals such as immediate, 6th and 12th hours. At the same time, it was aimed to shed light on clinical interventions against not only M, but also other lipofilic toxic substances by this study.

Materials and Methods

Animals, Care, and Nutrition

Forty-eight mature male Wistar albino rats weighing 200-250 g were randomly divided into six groups of eight rats each. The animals were kept under laboratory conditions with a 12-hour light/dark cycle and a room temperature of 21±3ºC. The Selcuk University Experimental Medical Research Center's Experimental Animals Ethics Committee approved the study.

Animals and Treatment

Forty-eight rats were randomly divided into six groups including; control (C), group (18.6 mL/kg oral IFE), Malathion group (10 mg/kg oral malathion), M0L group (IFE treated after immediate from malathion), M6L group (IFE treated after 6 hours from malathion), M12L group (IFE treated after 12 hours from malathion). After the administration of the medication, the animals were anesthetized with ketamine (50 mg/kg via intra peritoneal [ip]) + xylazine (5 mg/kg dose ip) and then sacrificed. All brain tissues taken from were evaluated in terms of biochemical and histopathological parameters

Biochemical Analysis

Brain tissue samples were used for determination of TAC (total antioxidant capacity) and TOS (total oxidant status) levels. They were weighed and cut into small pieces. Brain tissues were homogenized in 10 volumes of ice-cold phosphate buffer solution (50 mM/L, pH 7.0) using a homogenizer (Ultra-Turrax T8 dispersing homogenizator, Staufen, Germany). The homogenate was centrifuged at 15.000 rpm for 10 min at 4 °C to obtain supernatant. Supernatant samples were used for the determination of TOS and TAC levels. All biochemical analyses were performed in Biochemistry Department of Dicle University Medical Faculty.

Immunohistochemistry

The coronal brain sections were prepared for assessment of caspase-3 activity using an immunohistochemical staining procedure that was carried out by deparaffinization, dehydration and incubation in citrate buffer. A labelled streptavidin-biotin-peroxidase (immunoenzymatic) antigen detection system and AEC chromogen were used. The anti-activated caspase-3 primary antibody (1/100,Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA) was then applied in the blocking solition and incubated at 4°C overnight. The sections were biotinylated with goat anti-rabbit antibody (Santa Cruz Biotechnology Inc) in 0.1% PBST for 40 minute. Tissue section were colorized with SensiTek HRP (Anti-polyvalent) (ScyTek Laboratories, Logan,Utah,USA) for 8

min and counterstained with modified hematoxylin. Negative control sections were incubated in blocking solution that did not contain primary antibody. Prepared tissue were observed by a histopathologist unaware of the experimental study groups. The number of caspase-3 positive cells were counted in ten randomly selected microscope fields under a 200x magnification in a blind fashion. We calculated the average number of stained neurons for each set of ten fields and expressed as the number of the positive cells/high-power field.

Statistical Analysis

The data for the biochemical parameters were analyzed by ANOVA, followed by the post hoc Tukey test. All data was presented as mean ± SE using SPSS Windows 16.0 (SPSS Inc., Chicago, IL, USA). A value of $p < 0.05$ was considered statistically significant.

Results

Biochemical Results

M group in comparison with all others group, there was an increase in the TAC level. M group in comparison with C, L, M0L groups, it was seen significantly decrease in the TAC levels. Interestingly, M group in comparison with M6L and M12L groups, there was no significant difference among these groups in terms of the TAC levels. Although there was no significant difference among C, L and M0L groups in terms of both TAC and TOS levels, but was significant difference C, L groups in comparison with M6L and M12L groups in terms of TAC levels. C group in comprasion with L, M0L, M6L, M12L groups in terms of TOS levels, there was no significant difference. These results have indicated that IFE seriously reduced TOS levels in all the groups depending on time. Also, M0L group in comparison with M6L and M12L groups, there was significantly increase of the TAC levels. There was no statistically significant difference between M6L and M12L groups (Table I). These biochemical results were confirmed with immunohistochemical results.

Immunohistochemical Results

The biochemical results were confirmed by immunohistochemical appearance (Figures 1, 2, 3, 4). The number of caspase-3-positive brain cells in M, M6L and M12L group was higher than the others groups respectively (p = 0.001 for each).

Discussion

In recent years, has been reported that the lipid emulsion could reduce the toxic effects of fat-soluble drugs (clomipramine, propranolol, bupropion, haloperidol, organophosphates) poisoning by confining of the toxic substances or the chelating. Even though it has become almost a standard procedure and its usage is getting increased, the detailed mechanism of its action as an antidote is unknown[8, 9].

Previous studies have provided certain evidences that IFE is a promising theraupeutic strategy for fat-soluble drug poisoning. For example; Tebbutt et al[10] have stated that intralipid treatment prolongs survival and doubles median lethal dose in a rat model of verapamil toxicity, and the mechanism of action remains unelucidated. Zimmer et al[11] announced that treatment with lidocaine, propofol, and a 20% lipid emulsion resulted in fast resolution of cardiotoxic and neurotoxic effects in a case of suspected bupivacaine intoxication due to intravascular injection via an epidural catheter. Also, Weinberg et al[12] published data indicating that IFE is effective in treating of severe cardiotoxicity secondary to intravenous overdose of local anaesthetic drugs such as bupivacaine in a experimental model. Recently, some case reports have been published on the successful use of IFE for patients who were unresponsive to the usual resuscitation methods, and declared that all patients were recovered completely soon after intravenous injections of lipid[5,6]. Megerbane et al[13,14] stated that IFE improves Glasgow Coma Score, and decreases blood glucose level in the setting of acute non-local anesthetic drug poisoning.

These effects of intralipid is considered to be accomplished by reducing the amount of circulating lipophilic toxic drugs and its levels in target organs eventually. Only a few clinical studies have performed for the effect of lipid emulsions on the plasma concentrations of local anesthetics. Mizutani et al[9] reported that a case with ropivacaine-induced central nervous system toxicity treated successfully by lipid emulsion. After infusion of lipid emulsion, the ropivacaine concentrations decreased to 1.72 and 0.05 lg/ml, respectively. The patient was discharged without any complications.

As difference from Mizutani et al[9], the present study was planned with a hypothesis that M tends to be sequestrated into lipid-rich tissues like brain, causing neurotoxicity. If M ingesting is followed by intralipid througth the digestive

Table I. SNOT-22 pre-operatory visit. 6 months. 1 and 2 years individual item and total scores.

Dependent Variable				Mean Difference (Group I-II)	Std. Error	Sig.
TAC	Tukey HSD	K	M	.19400*	.01944	.000
			L	.00645	.01899	.999
			M0L	-.03200	.01944	.572
			M6L	.14433*	.01997	.000
			M12L	.17100*	.01861	.000
		M	L	-.18755*	.01899	.000
			M0L	-.22600*	.01944	.000
			M6L	-.04967	.01997	.146
			M12L	-.02300	.01861	.817
		L	M0L	-.03845	.01899	.342
			M6L	.13788*	.01953	.000
			M12L	.16455*	.01814	.000
		M0L	M6L	.17633*	.01997	.000
			M12L	.20300*	.01861	.000
		M6L	M12L	.02667	.01917	.732
TOS	Tukey HSD	K	M	-86.60800*	12.91897	.000
			L	19.79945	12.62194	.622
			M0L	7.98700	12.91897	.989
			M6L	1.74067	13.27298	1.000
			M12L	-14.34267	12.36897	.854
		M	L	106.40745*	12.62194	.000
		M0L		94.59500*	12.91897	.000
		M6L		88.34867*	13.27298	.000
		M12L		72.26533*	12.36897	.000
		L	M0L	-11.81245	12.62194	.935
		M6L		-18.05879	12.98405	.732
		M12L		-34.14212	12.05840	.067
		M0L	M6L	-6.24633	13.27298	.997
		M12L		-22.32967	12.36897	.471
		M6L	M12L	-16.08333	12.73827	.804

*The mean difference is significant at the 0.05 level.

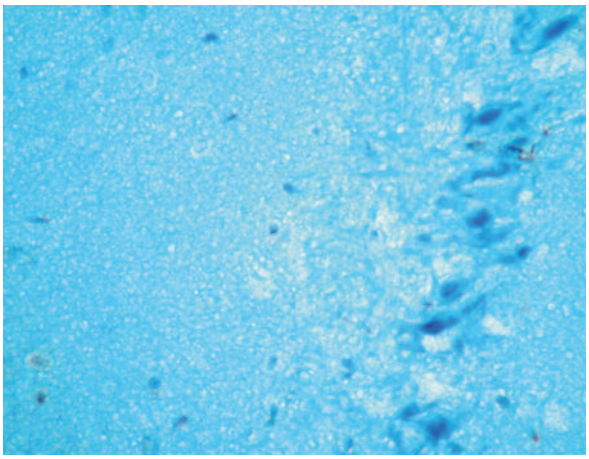

Figure 1. The control group no caspase-3 immunoreactivity (cell death/apoptosis is not seen) with normal brain tissue histology (Immunperoxidase/IHC x400).

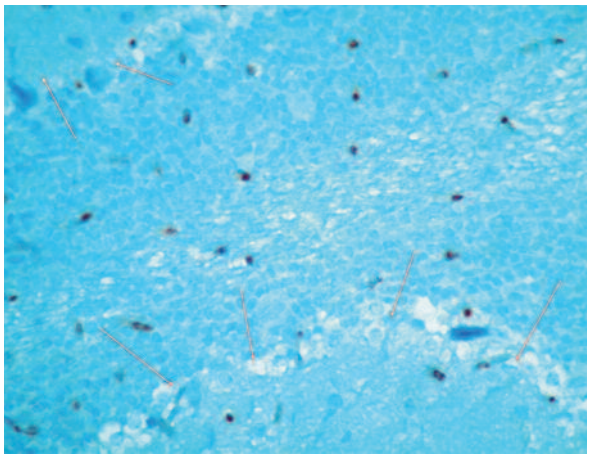

Figure 2. Malathion group. The tissue with the increased apoptosis index reflecting caspase-3 cell death and necrotic tissue in the area shown by the arrows (immunperoxidase/ IHC x400).

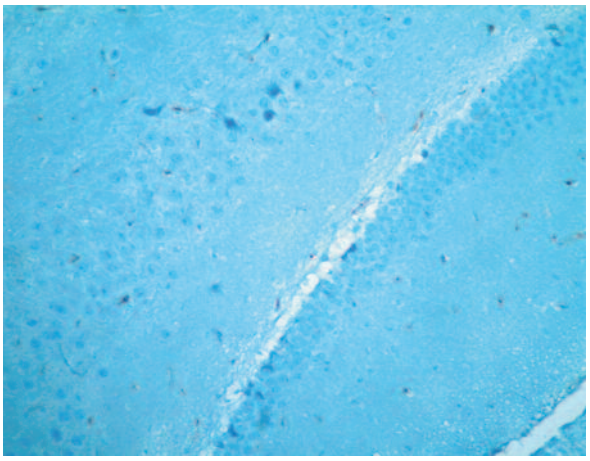

Figure 3. M0L group. The tissue sample with low caspase-3, apoptosis index (immunperoxidase/IHC x400).

tract and reduced the absorption of toxic substance and its the level of circulating, so that it is also expected to reduce the end organ toxicity.

As the mentioned above, IFE has been used to treat lipophilic drug ingestions, and theoretically may be also beneficial for some OP agents. Since most of organophosphorus pesticides are highly fat-soluble and, they can be stored in fat tissue. The concentration of OP pesticides in the fat tissue is found to be 20-50 times higher than those in the blood; therefore, IFE has the potential in treatment of OP poisoning[13].

Although therapies exist for acute OP exposure, but mortality rates remain high (10% to 20%). Limited previous work has been published on the effect of IFE during OP toxicity. For example; Bania et al[16] investigated the use of IFE on paraoxon (the active metabolite of parathion) toxicity in mices. While their results failed to demonstrate an increase in the LD50 of paraoxon with the use of IFE, it is possible that the extreme rapidity of paraoxon's toxicity. Han et al[17] reported that a case of glyphosate-surfactant herbicide (GlySH) poisoning with the shock that was refractory to vasopressors but responsive to IFE, and declared that IFE should be considered in case of refractory hemodynamic instability caused by GlySH in addition to- aggressive fluid and vasopressor support.

Dunn et al[4] published data on the animals demonstrating apnea and respiratory arrest by exposure of oral parathion at 4 × LD (50) doses. They are reported that IFE given immediately after oral parathion does not prolong the time to apnea. But IFE given 20 minutes after oral parathion intake decreases the acute effects of the OP and prolongs the time to apnea. The main difference of our study hypothesis from the literature studies is to investigate the effectiveness of intralipid given orally in different interval, and to determine the difference between at the level of the stomach (M0L group) and circulating OP (M12L group). Thus, the study will contribute to the strategy of time-dependent approach on the poisoning cases brought to the emergency room. For this reason, the present study shed light on the practical use and is an original work.

There is wealth of study pertaining to on M to cause to the oxidative stress in plasma and various organs, even its low doses[18-20]. In our study, similar to the literature, M group in comparison

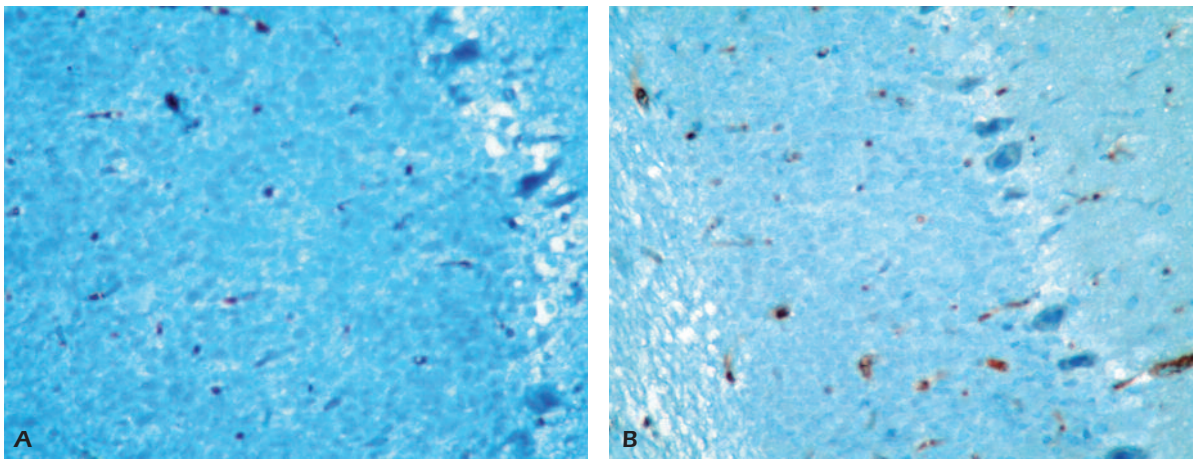

Figure 4. ***A-B***, M6L and M12L group. The tissue samples in which are the similiar Caspese-3 immunoreactivity intensity to M group (immunperoxidase/IHC x400).

with the other groups, it was determined that significantly increase in the TOS levels. M group in comparison with C, L, M0L groups, it was found that significantly reduction of the TAC levels. Interestingly, M group in comparison with M6L and M12L groups, there was no significant difference in terms of the TAC levels. Also, C group in comprasion with L, M0L, M6L, and M12L groups in terms of TOS levels, there was no significant difference, which indicated that L reduced TOS levels depending on time in all groups. Also, M0L group in comprasion with M6L and M12L groups, it was determined that significantly increase of the TAC levels. There was no difference significant between M6L and M12L groups. These biochemical results were confirmed with immunohistochemical results.

Conclusions

IFE is a promising safe therapy for the M or OP intoxication, and can be applied in the clinical practice. It would be more effective if used in the acute period of severe organophosphate poisoning.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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