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ORIGINAL ARTICLE

BLOOD-FETUS PENETRATION OF PRALIDOXIME

Shreesh Ojha[1], Syed M. Nurulain[1,2], S. Dhanasekaran[1], Mohammad Shafiullah[1], Abdu Adem[1], Charu Sharma[3], Kamil Kuca[4], Huba Kalasz[1,5]✉

[1] Department of Pharmacology and Therapeutics, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates
[2] COMSATS Institute of Information Technology, Islamabad, Pakistan
[3] Department of Internal Medicine, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates
[4] Centre of Advanced Studies, Faculty of Military Health Science, Trebesska 1575, 500 01 Hradec Kralove, Czech Republic
[5] Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, Semmelweis University, Budapest, Hungary

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Summary

Pralidoxime (2-PAM) is a monopyridinium aldoxime-type compound of acetylcholinesterase reactivators. 2-PAM was introduced about five decades ago for the treatment of organophosphorus poisoning in order to reactivate inhibited acetylcholinesterase. The application of organophosphorus compounds is varied, including warfare agents, insecticides and pesticides in agriculture, the chemical industry, etc. The exposure is not limited to certain groups of humans: rather everyone can be affected, including pregnant women, and consequently fetuses as well.

The present study was aimed to determine the 2-PAM concentration in the plasma of pregnant mice, assuming a different physiological condition than non-pregnant ones. Blood-placenta penetration of 2-PAM was also investigated. 2-PAM was intraperitoneally injected into mice on gestational day 18 and mother blood was collected following 5, 15, 30 and 90 minutes. Four fetuses along with their placentas were collected at every time point. HPLC-UV method was employed to determine the 2-PAM concentrations. The result showed higher levels of 2-PAM at 15 minutes (t_{max}) in the plasma of pregnant mice compared to non-pregnant ones.

Moreover, 2-PAM copiously reached the placenta, which is a store house of nutrients for the fetus. A higher concentration of 2-PAM was found in the brain of fetuses in comparison to that of the mothers'.

Our study concludes that 2-PAM crosses the placenta barrier and reaches the brain of the fetus in a more ample quantity than that in the mother's brain. The results provide an insight into a special condition of pregnancy when antidotal application of the acetylcholinesterase reactivator 2-PAM in organophosphorus poisoning results in 2-PAM exposure in the fetus.

Key words: Blood-fetus penetration; Mice; Pralidoxime; CNS penetration; Blood-placenta transfer

✉ Semmelweis University, Faculty of Medicine, Department of Pharmacology and Pharmacotherapy, H-1085 Budapest, , Üllői út 26, Hungary

INTRODUCTION

Pralidoxime (2-PAM, Figure 1) is the gold standard to treat organophosphate poisoning, generally

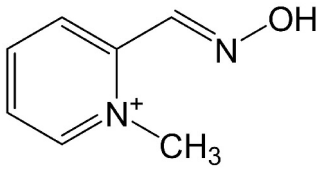

Figure 1. The chemical structure of pralidoxime.

as part of a mixture of other components, abbreviated as AFLOP: atropine, fluid, oxygen, pralidoxime. A heated discussion used to take place about its fate in the body. The major question was whether pralidoxime does or does not penetrate into the central nervous system. Bodor et al [1, 2] questioned penetration of pralidoxime through the blood-brain barrier, and suggested substitution of pralidoxime by its dihydropyridine derivative. Firemark et al [3] determined 2-PAM-C^{14} level in many body compartments, including several brain regions (cerebral cortex, white matter, central medulla and pons, caudate nucleus, inferior colliculi, hippocampus, etc.). The essence of their results [3] was confirmed by Sakurada et al [4], who used *in vivo* experiments on rats. Pralidoxime was given intravenously, and striatal dialysate samples were collected. Pralidoxime content was determined using HPLC analysis. Csermely et al [5] found dose-dependent penetration of pralidoxime into the brain, and also into the cerebrospinal fluid of rats. If the dose of pralidoxime was below 20 μM (i.p.) in rats of 200 g, a higher percentage penetrated into the central nervous system than that over this dose [6]. Kalász et al [7] compared blood-brain penetration of several mono-pyridinium and bis-pyridinium aldoximes. At the same time, several questions remained unanswered [8] concerning the mechanism of pralidoxime transport to the central nervous system and which parts of the brain are the targets of this transport.

The subject article is of utmost significance, since the experimental data on the fetus or placenta penetration of pralidoxime are scanty. Only two approximately related studies can be found. Lauder et al [9] reported that oximes reached the placenta and greatly reduced the teratological effect produced by OPC in chicken embryo though the efficacy was varied at different doses. There are ample studies indicating that OPC crosses the placenta barrier and affects the growing embryo [10-13].

There is no evidence to signify the quantity of oximes to cross the placenta barrier in fetuses. It is well established that several physiological, biological and endocrine changes occur during pregnancy which might influence the efficacy of acetylcholinesterase reactivator.

This paper is probably the first to demonstrate and quantify pralidoxime in pregnancy, deter-mines the passage of pralidoxime in the placenta and fetuses in a mouse model. The results will highlight the distribution of pralidoxime during pregnancy influenced by physiological conditions, an issue completely neglected by researchers but requiring immediate attention as the use of anticholinesterase pesticides is increasing globally.

This paper deals with how the distribution of pralidoxime changes in the body during pregnancy in mice, and how much pralidoxime is incorporated in the placenta and also in the brains of mouse fetuses.

MATERIALS AND METHODS

All chemicals and solvents were obtained from the commercial sources in the best available quality.

Experimental animals:

Studies were conducted following the approval of the United Arab Emirates University Ethics Committee (IAEC/13/03). The TO mice were originally purchased from Harlan Olac (England) and in-house bred. Virgin females were 6 weeks old, weighing 27-29 g. They were mated with males of the same origin in the evening. Vaginal plugs were found next morning that indicated the start of gestation day (GD0). The animals were housed in rooms of controlled light conditions (12:12 h). The average body mass of GD18 pregnant mothers, mother brains, pups' brains, placentas and non-pregnant mice is given in Table 1.

Mice (n = 4 or 5) were intraperitoneally (i.p.) treated with 40 mg/kg pralidoxime chloride freshly

Table 1. The average body weight/tissue weight of GD18 pregnant mothers, placentas and pups' brains and of pregnant mice.

Non-Pregnant mice body mass (g)	Pregnant mice body mass (g)	Pregnant mother brain mass (g)	Placenta mass (g)	Pup's brain mass (g)
Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
29.19 ± 2.63	46.58 ± 4.08	0.425 ± 0.020	0.108 ± 0.010	0.056 ± 0.007

dissolved in double-distilled water on the GD18. The mice were sacrificed after 5, 15, 30 and 90 min following treatment, and plasma, maternal brains, pups' brains and placentas were dissected and collected for studies. The samples were stored at −80 ºC before determination of their pralidoxime content.

Methods:

0.2 ml of 10% trichloroacetic acid was mixed to aliquots of plasma (0.5 ml) the precipitate was removed using centrifugation (5,000 rpm for 5 min at 4 ºC) and the supernatant was injected into the HPLC system.

Both the whole brain or about 100 mg of placenta tissue samples were weighed (wet), homogenized (T50 Ultra Turrax tissue homogenizer, IKA®-Werke GmbH & Co. KG, Germany) with 0.45 ml of phosphate buffer at 10,000 RPM under ice bath for 2 mins. Further on 0.3 ml of phosphate buffer and 0.2 ml of 10% trichloroacetic acid were added and centrifuged (at 5,000 rpm for 5 min at 4 ºC). The samples of supernatants were stored at -80 ºC before determination of their pralidoxime content, and were used for HPLC analyses.

Collection and processing of brain and placenta tissues: The entire brain was dissected; blood was removed, weighed, and frozen at -80 ºC. The same procedure was employed for pups' brains, and placenta tissue processing.

Instrumentation:

The HPLC system was a 717-Plus Autoinjector, a 515 HPLC pump, and 996 Photo Diode Array (PDA) detector. The separation was monitored at 286 nm, and evaluated using Empower ProSoftware (Waters Corporation, Milford, MA, USA). The stationary phase was Supelcosil LC-8 column (25 cm x 4.6 mm, 5 µm) purchased from Supelco, Bellefonte, PA, USA. The used mobile phase was 8% of methanol and 92% of 15 mM phosphate buffer (at pH 2.6 after adjusted with phosphoric acid). It also consisted 1 mM of 1-octane sulfonic acid sodium salt monohydrate. The flow rate was 1 ml min^{-1}. Separations were done at 26 °C.

Calibration for plasma and tissue measurement was done according to Lorke et al [9]. In each case calibration lines were constructed using plasma spiked with pralidoxime. The stability of each sample during the day was satisfactory.

Measurement of spiked pralidoxime concentrations in plasma, brain and placenta samples were treated in the same way as calibrators. The same volume was injected into the system and the peak area was determined. The concentration of pralidoxime was read against the corresponding concentration of the calibration curve.

RESULTS & DISCUSSION

Following the proper treatment of the appropriate body compartments of mice, pralidoxime spiked samples were subjected to HPLC following which they showed well-separated distinct peaks (Figure 2).

Pralidoxime concentration in control (non-pregnant) plasma, that of the plasmas, brains, placentas of pregnant mice and in pups' brains are given in Table 2.

The pregnancy of mice effects essential alterations in the kinetics of pralidoxime. The relatively sudden decrease in pralidoxime in the plasma of pregnant mice is the consequence of its incorporation in the placenta. The placenta shows an essential barrier to the defense of the fetus. At the same time, the placenta serves as a secondary source of pralidoxime in the body of pregnant mice.

The distribution difference between control and pregnant mice causes a striking change in the pralidoxime level of the brain. The mean pralidoxime level in pups' brains means a 5 to 50 fold increase relative to that in the mothers' brain (Table 2).

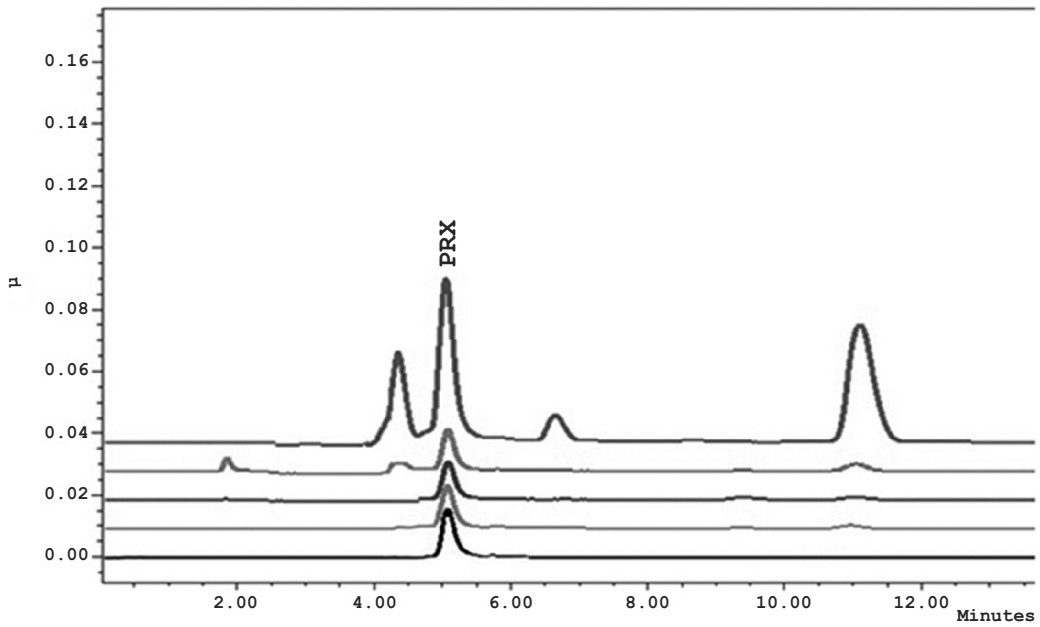

Figure 2. All pralidoxime spiked samples gave well separated distinct peak when they were subjected to HPLC. The chromatograms are given, from the top to bottom: placenta sample spiked with 2PAM, control brain sample spiked with 2PAM, pup's brain sample spiked with 2PAM, pregnant mouse brain sample spiked with 2PAM, standard pralidoxime

Table 2. Pralidoxime levels in non-pregnant plasmas, pregnant plasmas, placentas and pups' brains of mice. Values are given in µg/g tissue ± SD

Time after treatment (minutes)	Non-pregnant plasma	Pregnant plasma	Pregnant mice brain	Placenta	Pup's brain
5	6232.80 ± 1913.60	4689.13 ± 639.54	3.08 ± 1.14	591.48 ± 45.66	17.09 ± 7.99
15	2215.86 ± 503.95	4366.88 ± 2218.2	7.87 ± 1.92	1571.20 ± 866.40	50.90 ± 20.63
30	817.17 ± 224.08	53.12 ± 12.11	6.56 ± 2.25	831.54 ± 113.05	39.36 ± 8.49
90	379.89 ± 169.00	6.75 ± 2.01	4.83 ± 0.94	107.11 ± 6.32	77.95 ± 18.06

The present study highlights the distribution of pralidoxime in the plasmas of non-pregnant and pregnant mice, and quantifies the concentration of pralidoxime in the plasmas, placenta and fetuses of mice by HPLC method. The results revealed a higher concentration of pralidoxime in the placentas of pregnant mice. Pralidoxime efficiently crossed the placenta barrier and reached the fetuses brains in quite large quantities. Pralidoxime is the most widely used and WHO-recommended oxime in the treatment of organophosphorus anticholinesterase poisoning. It is also evident from the literature that organophosphorus anticholinesterase compound crosses the placenta barrier. In a recent study by Vera et al [14], decreased levels of placental cholinesterase were found in the pulverization period of women living near farms, suggesting the exposure to OPC, which consistently reached the plasma.

On the contrary, pharmacokinetics or toxicokinetics data of pralidoxime in the maternal blood or tissues of fetuses are hardly available. Conversely, there is no *in vivo* study indicating the reactivation potency under altered pregnant physiological conditions except some case reports (Wyttenbach and Hwang [15], Karalliedde et al [16], Jajoo et al [17]). A recent overview by Nurulain [18] pointed out the shortcomings of this important issue. In the fifty years of the existence of this compound [19], only two approximately related *in vivo* studies could be marked out, which do not provide complete information either. Edery et al [20] reported that low doses of 2-pralidoxime mesylate (P2S) did not cross the placenta barrier but high doses and continuous infusion of P2S resulted in crossing the placenta barrier. No animal model is mentioned in the only abstract available. Some recent studies, higher penetration

of pralidoxime chloride is noted which is a contrast to what is reported by Edery et al [20]. However, the difference may be accounted for the different animal models as well as the chemistry of the compound and for the method of detection used in 1963 vs these days. Andersen and Barstad [21] found a significant penetration of tertiary and quaternary nitrogen compounds through the placenta of rats. Here again, we could not find complete papers, though our results correspond with Andersen and Barstad's [21], although we used monoquaternary aldoximes in mice. It is also noteworthy that in our experiments, pralidoxime was administered on the last day of pregnancy in mice (i.e. GD18). Administration on other days of gestation might yield different results, which need to be addressed in further studies. The study demonstrated the copious passage of pralidoxime in the placenta and in fetus' brain when pralidoxime was administered on GD18.

Breslin et al. [22] did not find developmental effects of chlorpyrophos. Organophosphorous pesticides (such as chlorpyrophos) are unable to produce gestational exposure to such an extent as they do it concerning non-pregnant and pregnant mothers, as it was experimentally proven by Lassitner et al [23] and Farag et al [24]. Excessive penetration of pralidoxime means that an expressed defense to the fetus is needed in cases of organophosphorous intoxication.

CONCLUSIONS

The present data warn that a definite protection to the fetus' brain is required when a pyridinium aldoxime is given during the defense following organophosphate intoxication. The dose of pralidoxime should be adjusted in case in pregnancy.

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Dr Ohja and Dr Nurulain should be considered as first authors.

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