

Characterization of Early Plasma Concentrations of Midazolam in Pigs after Administration by an Autoinjector

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ABSTRACT: The treatment of organophosphate-induced poisoning is based mainly on atropine and an oxime. Prompt anticonvulsive intervention is usually also required to terminate the ensuing seizure activity and to prevent delayed permanent brain damage. Midazolam, a water-soluble benzodiazepine agonist, has the advantage of rapid absorption following intramuscular administration. In mass casualty situations, the availability of an autoinjector, filled with midazolam, might be a further advantage. In the present study, the plasma pharmacokinetics of midazolam after administration by an autoinjector was compared with conventional intramuscular (i.m.) administration in two groups of four pigs each. During the first 15 min after injection, significantly higher plasma concentrations of midazolam were detected following autoinjector administration, compared with the i.m. injection. The physiological reflection of the accelerated midazolam absorption was a marked reduction in the time interval required for muscle relaxation, induced by midazolam. It is concluded that a midazolam autoinjector might be helpful in the mass casualty scenario following organophosphate poisoning. Copyright © 2004 John Wiley & Sons, Ltd.

Key words: anticonvulsants; benzodiazepines; organophosphates; sedation; seizures

Introduction

One of the most important elements for the successful treatment of organophosphate (OP) cholinesterase-inhibitor poisoning is its prompt application. While traditional routine therapy is based on the administration of the muscarinic blocker atropine and an oxime that reactivates the inhibited enzyme, rapid intervention with an anticonvulsant for the quick termination of seizure activity is also often required [1,2]. Inefficient or delayed anticonvulsive treatment may lead to permanent brain damage [3–5]. Diazepam (or its pro-drug avizafone) is used in the treatment strategy of various countries. Compared with diazepam, midazolam, a water-

soluble benzodiazepine agonist, has the advantage of a higher bioavailability and a more rapid absorption and onset of activity, following intramuscular (i.m.) administration [6]. Therefore, the drug has been suggested as a possible replacement for diazepam in the US army [7]. Midazolam has a further advantage when administered through the nasal route [8,9], although at this point, at least for battlefield application, this route does not seem practical yet. In a comparison between the various benzodiazepines for the control of soman-induced seizures in guinea-pigs, midazolam was found to be the most potent and rapidly acting [10]. Midazolam also had a better performance when compared with diazepam in monkeys [11].

A few papers in the literature reported the different pharmacokinetics of drugs following intramuscular administration using an autoinjector. In allergic children, epinephrine absorption

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was faster following injection with the automatic EpiPen injector [12]. The time to peak concentration was significantly reduced compared with subcutaneous administration, which in the case of anaphylaxis could be extremely important. The spring-loaded self-injector Atropen, containing the equivalent of 2 mg atropine sulfate, was studied in 15 healthy volunteers. It was found to produce a significantly earlier increase in heart rate compared with atropine in a conventional syringe injected i.m. [13]. In another study, the performance of an atropine autoinjector (Mark I, used in the US Army) was tested in 20 human volunteers against a different injector referred to as an MCP [14]. The Mark I injector exhibited a significantly higher atropine concentration in plasma 10 min after injection. The decrease in salivation was significantly more pronounced during the first 15 min and the increase in heart rate was statistically significant up to 30 min following administration. The more rapid changes were attributed to a broader dispersion of the drug in the injected tissue. A Swedish group compared the pharmacokinetics of HI-6 and atropine following injection with an Astra Tech autoinjector to anaesthetized pigs with that of conventional intramuscular delivery [15]. The time interval for obtaining the therapeutic concentration of HI-6 (defined as 4 µg/ml) was reduced from around 5 min to 1 min.

No data are available for the pharmacokinetics of midazolam administered by autoinjectors. The importance of prompt anticonvulsant administration after OP poisoning is the subject of the present study.

Materials and Methods

The study was approved by the IIBR Committee for Animal Experimentation, with emphasis on the prevention of any unnecessary animal suffering, the use of the smallest number necessary and was according to the recommendations of the *Guide for the Care and Use of Laboratory Animals*, National Academy Press, Washington DC, 1996. White domestic young piglets (supplied by Lahav, Israel), weighing around 12 kg, were used throughout the study, 2–3 days following their

arrival to the Institute's animal facility. Four comparable experiments were performed. For each experiment, one pig was injected with an autoinjector and the other by conventional needle and syringe, the pigs being matched by weight to a difference not greater than 100 g. Because pigs grow very fast, a cross-over design was not possible.

Autoinjectors, normally used for atropine, were supplied by Shalon, Israel, each containing 5 mg of midazolam hydrochloride in 0.7 ml of saline plus preservatives. For conventional i.m. injection, the content of some of these autoinjectors was released and measured. They were found to release consistent volumes (0.734 ± 0.002 ml $n = 5$). Thus, using a 1 ml syringe and 23G-1" needle (identical in size to the autoinjector needle), 0.73 ml of this midazolam solution was injected i.m. into the gluteus muscle as a single bolus. The animals were monitored visually, as well as physically by lifting their head, to determine muscle relaxation and sedation. An ANOVA test was performed on the results for the 'time to muscle relaxation', to determine whether the differences between the two groups were statistically significant.

One ml blood samples were withdrawn from the pig subclavian vein at 1, 2, 4, 7, 10, 15, 20, 30 and 60 min following injection, as well as after 2, 3, 4 and 5 h. Plasma was separated and kept frozen at -70°C until assayed.

Midazolam was determined by radio receptor assay (RRA), using an adaptation of Citron's method for scopolamine [16]. Briefly, 200 µl of standard midazolam samples in plasma (in the range 3.9–1000 ng/ml) were prepared and added to 5 ml of 0.05 M phosphate buffer (PB) pH 7.4. The diluted standard solutions, as well as the diluted study samples, were loaded on activated Sep-Pak C18 columns (Waters, Milford MA), washed with 5 ml PB and then with 5 ml of double distilled water containing 20% v/v methanol. The drug was extracted from the columns with 5 ml of 100% methanol, and dried overnight under vacuum and centrifugation. The samples and standards were resuspended in 200 µl of PB and 25 µl in duplicates were added to the competitive binding mixture. [N-Methyl- ^3H]-Flunitrazepam (TRK-590, Amersham UK), having a specific activity of 96 Ci/mmol, served

as the radioactive ligand in the assay, in which midazolam competed for its binding to rat brain membranes, as described previously [16]. The minimal concentration of midazolam in the standard curve, i.e. 3.9 ng/ml, determined the limit of sensitivity of the assay as seen in Figure 1. Since standard solution samples were always processed concomitantly with the study samples in the same assay, and spiking of standards in plasma gave similar results, the recovery of midazolam from standards and samples was considered identical. To determine the statistical significance of the differences between the two modes of injection, an ANOVA test was employed and the individual AUC values for each animal were compared between the two groups.

Results

Midazolam concentrations in plasma were determined at 13 time points following injection, in four pigs for each experimental group. The complete data are presented in Figure 2. According to the principles of pharmacokinetics, if the dose was indeed identical in the two groups, similar areas under the curve (AUC_{0-300}) should be obtained. The values obtained for the

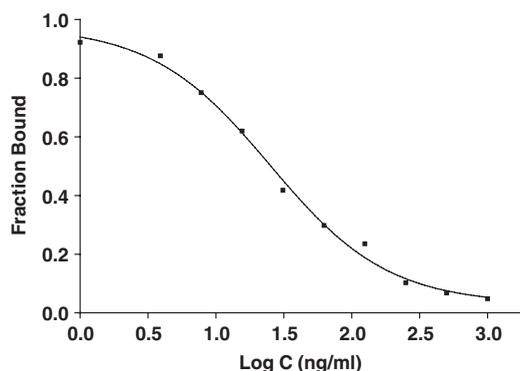


Figure 1. A representative standard curve for midazolam determination in plasma by radio receptor assay (RRA), in which it competed with the binding of ^3H -flunitrazepam to membranes from rat brain. The standard solutions went through a similar extraction procedure to that carried out on the samples

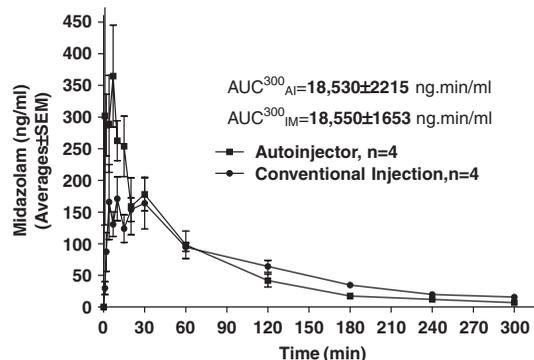


Figure 2. Pharmacokinetic follow-up on midazolam concentrations in plasma after autoinjector (AI) administration to pigs in comparison with conventional syringe and needle injection (i.m.). Four pigs were used in each group (average \pm SEM)

two curves, using GraphPad Prism software, were virtually identical (18530 ± 2215 and 18550 ± 1653 ng.min/ml). Using the pharmacokinetic relationship $\text{Dose} \cdot F = AUC \cdot CL_p$, where CL_p/F represents the total plasma clearance, a clearance value of 161/h was obtained for these young pigs (or approximately 1.31/kg.h).

Despite having similar AUC s, it was apparent that the two pharmacokinetic curves were not identical. To emphasize the differences, the data were expanded and presented separately for the first hour (Figure 3). During the first 15 min following injection, the AUC for the autoinjector group (4240 ± 343 ng.min/ml) was significantly higher than the value obtained for the conventional i.m. injection (1960 ± 346 ng.min/ml; $F(1,6) = 18.56$, $p < 0.005$ by ANOVA). The average value of the plasma concentration (293 ± 47 ng/ml) was also more than two times higher than that obtained following regular i.m. injection (118 ± 21 ng/ml). The ratio was reversed during the next 4 h, during which the AUC was higher for the regular i.m. administration.

During the experiments it became obvious that the pigs responded more rapidly to the same dose of midazolam when injected using the autoinjector. Measurements of the time interval required to achieve muscle relaxation, assessed by lifting the pigs' head, are summarized in Table 1.

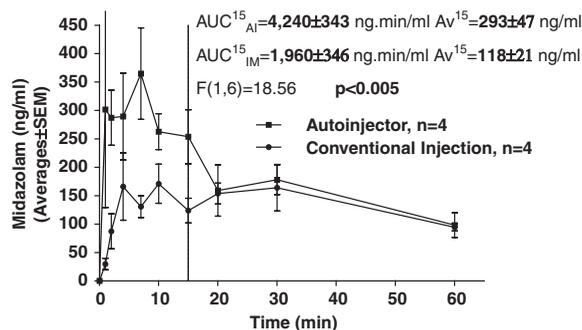


Figure 3. Expanded presentation of the pharmacokinetic curves in Figure 2, for the first hour following administration

Table 1. The effect of injection mode on the time interval for the onset of midazolam's physiological effects (AI, autoinjector; IM, conventional mode)

Injection mode	Onset of muscle relaxation (average in min \pm SEM) ^a	AUC until muscle relaxation (ng.min/ml, average \pm SEM)
IM	4.75 \pm 1.2	313 \pm 44
AI	2.5 \pm 0.3 ^b	578 \pm 191 ^c

^aSimilar effect was observed for onset of sedation.

^bDifference was statistically significant, $p < 0.03$ according to ANOVA test.

^cNot statistically different from the i.m. group.

Discussion

It has been reported that midazolam, as other benzodiazepine agonists when administered following OP poisoning, acts by activation of the inhibitory GABA system and therefore is still effective in suppressing seizures during the later stage of intoxication, which follows the immediate cholinergic stage [17]. Nevertheless, it is most efficient during the first 10–20 min following the beginning of the abnormal electrical brain activity. It is precisely during this time frame that an autoinjector might be able to contribute to higher plasma concentrations and thus to enhanced therapeutic activity.

The rapid and relatively efficient absorption of midazolam is its major advantage over other benzodiazepine agonists (e.g. diazepam). As previously stated, autoinjection was found to contribute to epinephrine absorption and, to a

certain extent, to HI-6 absorption. The use of an autoinjector containing midazolam increased significantly the plasma levels obtained during the first 15 min following administration and thus might contribute to its efficacy as well. This increase, similar to that found for other drugs in the past [12–14], is probably attributed to the larger area spread of the drug following autoinjector administration, which facilitates faster absorption. In a human study of 15 subjects with various midazolam doses, it has been found that the C_{max} of the drug was proportional to the administered dose, and the degree of sedation was significantly correlated to the midazolam plasma levels [18].

Special attention was devoted to the experimental procedure of the present study to match the two groups (e.g. similar needles, similar pigs' weight, similar midazolam doses and similar injection volumes) to avoid possible experimental bias. Indeed, the AUCs of the pharmacokinetic curves for the two experimental groups were identical, thus proving that a similar dose was absorbed and that the effect could be related to the injection mode. From the AUC value (0.31 mg.h/l) and the dose (5 mg) it is possible to estimate the value of CL_p/F in these young piglets (161/h or 1.31/h.kg). In human adults, a total clearance value of 181/h was reported for midazolam and a value of 0.551/h.kg for children [19]. The biotransformation of midazolam occurs primarily in the liver (through the enzyme cytochrome P450 3A4) and it is excreted mostly as a glucuronide in the urine. Inter species differences such as those reported here are reasonable and may represent differences in enzymatic activity and liver blood flow.

An attempt was also made to match the area under the curves up to the point of onset of the physiological effect in the two groups (see Table 1). However, the rapid increase in plasma concentrations following administration by an autoinjector, coupled with the scanty number of blood samples immediately following injection are probably responsible for the large variation in AUC values for this group.

In summary, the significant increase in midazolam plasma concentrations following administration by an autoinjector found in this study during the first 15 min following administration,

as well as the significant shortening of the time to onset to muscle relaxation, indicate that the use of the autoinjector may improve the efficiency of midazolam in the rapid termination of seizure activity after OP poisoning.

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