Toxogonin: Oral Administration to Man

FREDERICK R. SIDELL and WILLIAM A. GROFF

Abstract □ Toxogonin, administered orally in an aqueous solution to healthy male volunteers, produced dose-related blood levels of oxime. These levels were lower than those found with similar doses of 2-pyridinium aldoxime methochloride. A dose-independent symptom complex of facial warmth and/or numbness and a cool menthol sensation in the throat was similar to, but of less intensity than, that previously seen when toxogonin was given intramuscularly. If toxogonin shows further therapeutic promise, additional investigations into methods to facilitate its gastrointestinal absorption are indicated.

Keyphrases □ Toxogonin, oral administration—dose-related blood, plasma levels, oxime □ Absorption, toxogonin—oral administration □ Dose-independent symptoms—toxogonin □ Urinary excretion—toxogonin

Toxogonin [*N,N'*-oxydimethyl(pyridinium-4-aldoxime) dichloride] has been suggested as a replacement for pyridine-2-aldoxime methochloride as the oxime of choice in the therapy of anticholinesterase poisoning (1, 2). *In vitro* studies indicated that toxogonin is 15–30 times more effective than pralidoxime in reactivating cholinesterase inhibited by certain anticholinesterases (1, 2); animal studies showed it to be more effective (3–5), although not quite as much as suggested by *in vitro* studies. In cases of poisoning in man, it was more effective than (6), or at least as effective (7) as, pyridine-2-aldoxime methochloride.

By intramuscular administration to men, the dose of toxogonin necessary to produce plasma levels above 5 mcg./ml. is about one-third the dose of pyridine-2-aldoxime methochloride needed for similar levels (8), although the significance of this is not known since a therapeutic blood level has not been determined. Another point in its favor is that toxogonin produced hypertension in unpoisoned men (8), which is a possible benefit in a severely poisoned individual whose circulation is failing.

Although oxime should be given to a severely poisoned individual by intravenous injection, oral therapy might be useful in some circumstances. One instance might be in poisoning by a substance that is rather inactive *in vitro* but is metabolized (or "toxified") in the body to a toxic anticholinesterase; *e.g.*, parathion to paroxon. In this case, it is desirable to maintain a constant level of oxime over a period of time, which would require repeated injections if parenteral administration was used. A recent report describes the effectiveness of pyridine-2-aldoxime methochloride administered in this manner to poisoned rats (9).

Oral oximes also might be given prophylactically (10, 11) to men with a high risk of exposure to anticholinesterases, such as crop dusters. Finally, oral administration of oximes might be considered for a poisoned individual whose signs and symptoms are very mild, not requiring urgent treatment.

To assess how well toxogonin is absorbed when administered orally to men as determined by blood levels of oxime and to investigate the side effects caused by toxogonin administered orally were the purposes of this study.

SUBJECTS, METHODS, AND MATERIALS

The subjects were U. S. Army enlisted men between the ages of 19 and 31 years (mean 21.6) who volunteered for and were accepted into this study after a physical examination, chest X-ray, electrocardiogram, and laboratory tests[1] revealed no abnormalities. They weighed from 66.8 to 98.4 kg. (mean 78.1). They were told they were to receive a new and possibly useful antidote for anticholinesterase ("nerve agent") poisoning and that they might experience some symptoms (left unspecified). Their only reward for participating was a day off from duty.

Toxogonin was purchased[2]. Spectroanalysis showed it to be over 98% pure; the intraperitoneal LD_{50} [by the method of Bliss (12)] in 15–20-g. female mice was 109.6 (95% CL: 102.5–117.2) mg./kg.

Three or four subjects participated each test day. On each morning of the test, a fresh 25% (w/v) solution of toxogonin in distilled water was prepared and administered orally within 1 hr. of preparation. The doses selected were 1, 3, 5, 7, and 9 g.; the volumes of toxogonin solution were correspondingly 4 to 36 ml. The lowest dose and the highest dose were given to two subjects each; the other three doses were given to three subjects each.

Subjects were admitted to the test ward on the evening before the test. They were allowed no solid food from midnight until 3 hr. after they had taken the drug, which was about 8 a.m. A high fluid intake was urged from the time they awoke (about 6:30 a.m.) until the conclusion of the test.

In the hour before the drug was given, three measurements of blood pressure and heart rate were made with the subject supine. These measurements were repeated hourly for 6 hr. after drug administration. Also during this period, blood was drawn for control laboratory studies (hematocrit, hemoglobin, total and differential white blood cell counts, blood urea nitrogen, alkaline phosphatase, and serum glutamic oxaloacetic transaminase) which were repeated 1 and 7 days later. Each subject voided before receiving the drug; this urine specimen was used as a control for oxime analysis and urinalysis.

In three subjects (all 7-g. dose), leukocyte chromosomes were studied before the subjects received toxogonin and 4 hr. and 7 days afterwards. The preparations were made by the method of Moorhead *et al.* (13).

Blood was obtained at 0.5, 1, 2, 3, 4, 5, and 8 hr. after drug administration; the whole blood and plasma were analyzed for toxogonin content by methods previously described (14). The oxime content of the red cells was also measured and will be the subject of a separate report. Urine was collected and analyzed for oxime content (14) at hourly intervals for the first 10 hr.; thereafter, for 24 hr., all urine was collected and analyzed, but no time schedule for collection was imposed. Most of the urine specimens were refrigerated overnight and analyzed. A preliminary study, performed by adding a known amount of toxogonin to urine and analyzing aliquots daily, had shown that there was less than 5% loss of toxogonin over a week.

[1] Hematocrit, hemoglobin, total and differential white blood cell count, urinalysis, blood urea nitrogen, alkaline phosphatase, serum glutamic oxaloacetic transaminase, bilirubin, total protein and albumin, and red blood cell and plasma cholinesterase.

[2] E. Merck, Darmstadt, Germany.

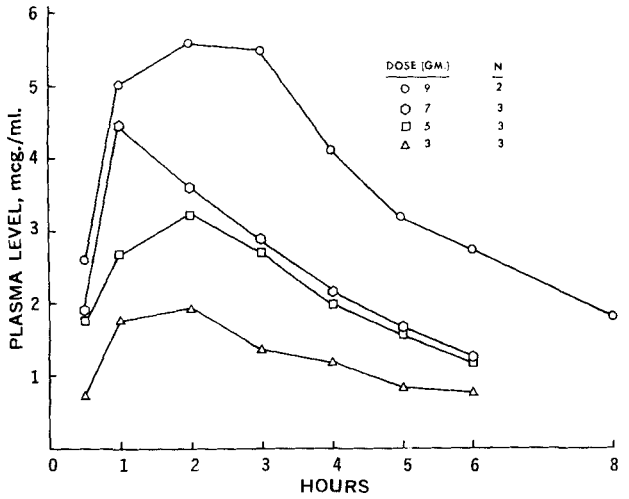

Figure 1—*Toxogonin: mean plasma oxime levels after oral administration.*

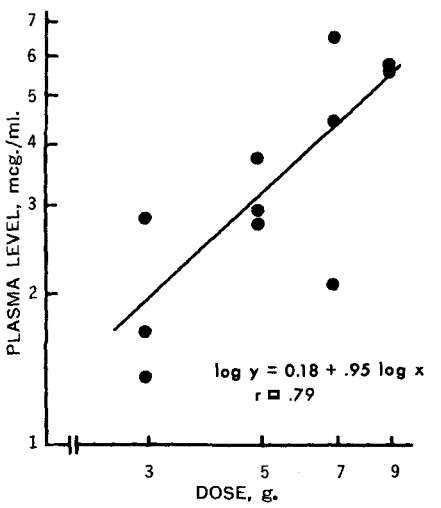

Figure 2—*Toxogonin* (per os)*: dose* versus *maximal plasma level.*

The subjects were allowed to be out of bed except for 10 min. preceding measurement of heart rate and blood pressure.

Because of the low amount of oxime excreted in the urine (see *Results*), it was thought that perhaps excretion was delayed. Three additional subjects were given toxogonin (two received 7 g. and one received 3 g.) under the same test conditions, except that blood was drawn at 2 and 3 hr. after drug only to ensure that toxogonin was present in about the same concentration as it was in the others; all urine was collected for oxime analysis for 48 hr. (These blood and urine values were not included in the tabulated results.)

RESULTS

Blood Levels of Oxime—The oxime plasma concentrations of the two subjects who received 1 g. of toxogonin reached maximum values of about 1 mcg./ml., but the lower values varied considerably. Since these levels are at the limits of the analytical method, these data were not tabulated.

Figure 1 shows the time courses of the mean plasma values for oxime for the other dose groups (through a laboratory error, the 8-hr. plasma value for three subjects, one at a dose of 5 g. and two at 7-g. doses, was discarded). The whole blood oxime concentrations were about 60–70% of the plasma values and are not shown. Maximal values were reached in 1 or 2 hr.; for all but the 7-g. dose group, a plateau level was maintained for the next 1–2 hr., undoubtedly because of continued absorption.

A plot of the dose *versus* the maximal measured plasma concentration for each subject is shown in Fig. 2.

In a previously reported study (15), conducted with similar subjects and test conditions, commercially available tablets of pyridine-2-aldoxime methochloride produced higher plasma levels than those found for toxogonin. Figure 3 shows these comparisons. The plasma concentration of toxogonin is roughly 50–60% of that produced by an equiweight dose of pyridine-2-aldoxime methochloride.

Urinary Excretion—Overall, 2.2% of the dose given was excreted in the urine during the 24-hr. collection period; percentage excretion by dose group was 1.4 for 3 g. (range 0.3–3.1), 2.4 for 5 g. (range 2.3–2.4), 2.2 for 7 g. (range 1.3–3.7), and 3.2 (range 3–3.4) for 9 g. This would not have increased over the next 24 hr.; the percentages excreted by the three subjects whose urine was collected for 48 hr. were 2.1 (7-g. dose), 3.5 (7-g. dose), and 0.7 (3-g. dose).

Half-Lives of Oxime—*Plasma*—By assuming that a drug is eliminated by first-order kinetics, the half-life for it in plasma ($t_p{}^1/_2$) and its rate constant of elimination (k_p) can be calculated from the slope of a line drawn through the terminal points when the plasma concentration is plotted on a logarithmic scale against time on a linear scale. For nine of the subjects, the coefficient of correlation of this line through the last five to six values was about 0.99; for one subject, the coefficient of correlation was 0.96; and for one subject (7-g. dose), the coefficient of correlation of such a line was poor and his data were not used in half-life calculations.

The mean plasma half-life of toxogonin for 10 subjects was 2.64 hr. (for 3 g., 2.41 hr.; for 5 g., 2.78 hr.; for 7 g., 2.29 hr.; and for 9 g., 3.10 hr.). The mean k_p value for the 10 subjects was 0.27 hr.$^{-1}$ (by dose group: 0.29 for 3 g., 0.24 for 5 g., 0.31 for 7 g., and 0.23 for 9 g.). This half-time is not significantly ($p > 0.1$ by t-test) different from the half-life of pyridine-2-aldoxime methochloride (mean 2.66 hr.) found in earlier studies.

Urine—The rate constant (k_u) and biological half life ($t_u{}^1/_2$) for urinary excretion can be obtained from the slope of the regression line of the plot of the amount to be excreted (total amount excreted over the entire collection period minus the amount excreted by time, t) as a percentage of the total amount excreted (on a logarithmic scale) against time, t (16).

The plots for the means of each dose group are shown in Fig. 4. The $t_u{}^1/_2$ values were 4.4 hr. for 3 g., 5.0 hr. for 5 g., 5.2 hr. for 7 g., and 4.6 hr. for 9 g., with a mean for all doses of 4.8 hr. Corresponding k_u values were 0.16 hr.$^{-1}$, 0.14 hr.$^{-1}$, 0.13 hr.$^{-1}$, and 0.15 hr.$^{-1}$.

This time is significantly ($p < 0.01$ by t-test) longer than the corresponding values for pyridine-2-aldoxime methochloride (mean of 2.44 hr.).

Other Laboratory Findings—There were no alterations in the values obtained in any of the routine laboratory tests at 1 or 7 days.

Physiological Effects—There were no significant changes in the heart rate or blood pressure of any subject.

Chromosome Studies—There was not an increased number of gaps or breaks, nor was there an increase in the mitotic index in the chromosomes of the three subjects (all 7-g. dose) in whom chromosomes were studied.

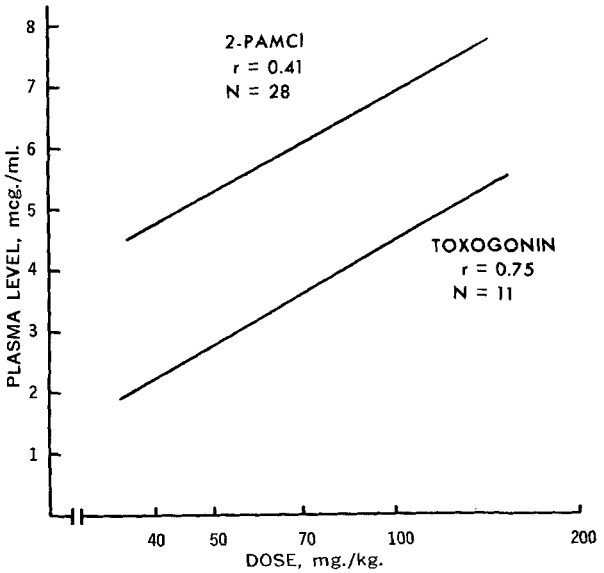

Figure 3—*Toxogonin and pyridine-2-aldoxime methochloride: comparison of plasma levels after oral administration.*

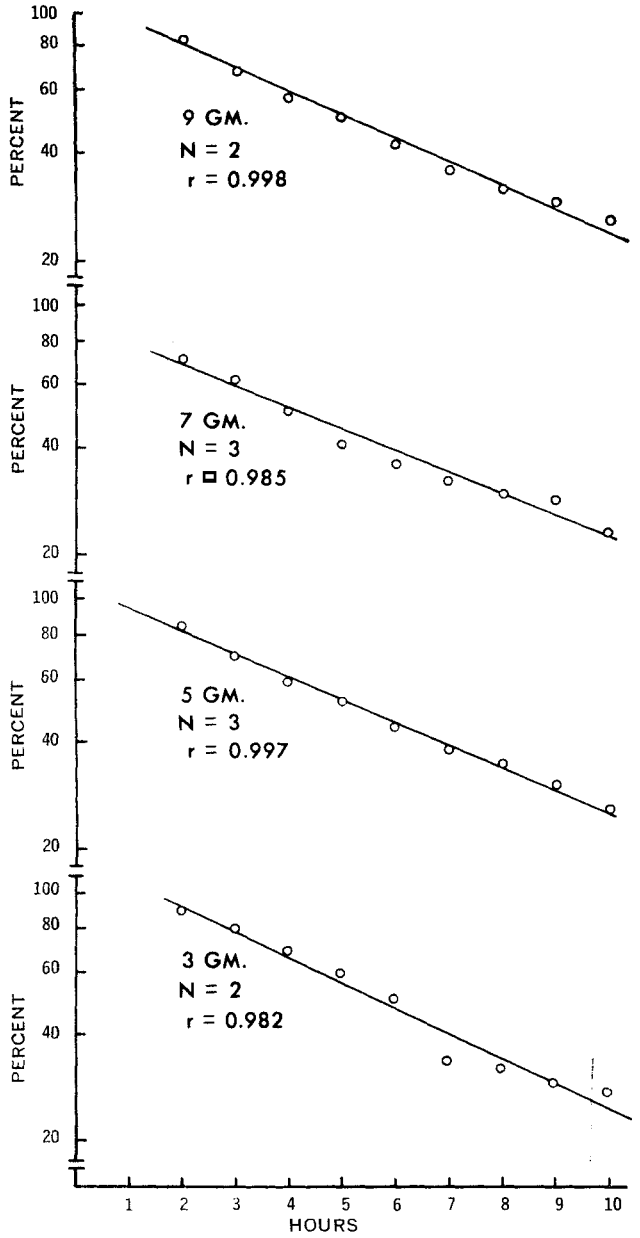

Figure 4—*Toxogonin: percent not excreted* versus *time.*

Side Effects—Most of the subjects described the taste of the solution as "bitter" or "salty" but tolerable.

The symptoms were similar to those reported after intramuscular toxogonin (*1, 8*), but their intensity was less. Two subjects receiving 3 g. noted numbness of their lips; one reported a cool sensation in his mouth, and one noted a dry mouth. At 5 g., one reported a cool sensation in his mouth, and one noted that his mouth felt dry. Of the five subjects receiving 7 g., two reported a tightness in their cheeks, four said their faces felt numb, and two spontaneously described a menthol taste and coolness in their throats. One subject at a dose of 7 g. became nauseated and vomited about 1.5 hr. after receiving the oxime. (This subject's oxime blood levels were among the highest in this dose group and were included in all analyses.) One subject receiving 9 g. said his face felt warm and numb. Several in each dose group complained of feeling drowsy or of their eyes feeling heavy.

DISCUSSION

Erdman and Okonek (17) reported that no measurable blood levels of oxime were found in three human subjects after the oral administration of 30 mg./kg. of toxogonin in a tablet also containing 24 mg./kg. of disodium ethylenediaminetetraacetic acid as an adjuvant. At a dose of 1 g. (about 12 mg./kg. in two subjects, each weighing 82 kg.) in the present study, maximum plasma levels of about 1 mcg./ml. were found, which is close to the limit of the analytical method. At higher doses, proportionally higher blood levels were found.

Orally administered toxogonin gives relatively low blood levels of oxime when compared to oral pyridine-2-aldoxime methochloride. Although, in general, an aqueous solution of a drug should be absorbed more quickly and completely than a drug compressed into a tablet which must undergo disintegration in the gastrointestinal tract before absorption, it is possible that toxogonin and perhaps other oximes are degraded by gastric acidity or other factors against which the tablet or capsule protects the active ingredient. Erdman and Okonek (17) demonstrated in rats that this may be true for toxogonin because the drug was more completely absorbed after instillation into the duodenum than from instillation into the stomach. This suggests that if it is in an appropriate pharmaceutical preparation, one resistant to gastric action, the gastrointestinal absorption of toxogonin might be greater.

The small amount of oxime excreted in the urine is perhaps a reflection of the poor absorption of toxogonin or could be due to the metabolism of the compound to a substance not detectable by this method. Since in a similar study on toxogonin administered by the intramuscular route, the authors found 82% of the compound in the urine (8), we tend to discard the latter suggestion, although the metabolism of a compound may be different when it is administered by different routes.

If it can be assumed that the amount of drug excreted in the urine is a direct reflection of the amount of drug absorbed, there is a disparity between plasma levels and excretion of toxogonin compared to 2-pyridinium aldoxime methochloride. For similar doses (milligrams per kilogram), the plasma levels of toxogonin are more than 50% of those obtained with 2-pyridinium aldoxime methochloride, but the percentage of the dose of toxogonin found in the urine is about one-fifth of that found after 2-pyridinium aldoxime methochloride (15), suggesting that less of the dose is absorbed. One possibility, as noted, is that toxogonin is metabolized to a compound not identified in the urine. Another possibility is that the volume of distribution (the hypothetical volume of body fluid in which the substance is present in the same concentration as it is in the plasma) is much smaller for toxogonin than for 2-pyridinium aldoxime methochloride, in which case higher blood levels would be produced by smaller amounts of drug. This latter possibility can only be verified by further work.

The exact therapeutic effectiveness that might be expected from these plasma concentrations is unknown, although evidence suggests that plasma levels of 2–5 mcg./ml. would be adequate. Sundwall (18), in *in vivo* studies, suggested that 4 mcg./ml. of the methanesulfonate salt of pralidoxime is adequate to reverse certain effects (*e.g.*, neuromuscular block) produced by an anticholinesterase. In *in vitro* studies, Erdman *et al.* (1) noted that 3 mcg./ml. of pralidoxime (exact salt not specified) was required to reactivate cholinesterase inhibited by paraoxon (a value close to that found by Sundwall *in vitro*), but only 0.1–0.2 mcg./ml. of toxogonin was needed to produce the same amount of reactivation under the same conditions. These latter investigators (1) also noted that cholinesterase depression produced in the blood of dogs and cats by 20 times the lethal dose of parathion was quickly regenerated by 1–3 mg./kg. of toxogonin, a dose which in man produces a plasma level of around 2–5 mcg./ml.

If, as suggested by Erdman and coworkers (1, 2), toxogonin is a better antidote than 2-pyridinium aldoxime methochloride for anticholinesterase poisoning, further investigations on mechanisms to facilitate intestinal absorption are indicated if oral administration is desirable.

SUMMARY AND CONCLUSIONS

Toxogonin administered orally in an aqueous solution to healthy male volunteers produced dose-related blood levels of oxime. These levels were lower than those found with similar doses of 2-pyridinium aldoxime methochloride. A dose-independent symptom complex of facial warmth and/or numbness and a cool menthol sensation in the throat was similar to but of less intensity than that previously seen when toxogonin was given intramuscularly. If toxogonin shows further therapeutic promise, additional investigations into methods to facilitate its gastrointestinal absorption are indicated.

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Kinetics of Rheological Properties of Acacia Solutions

DENNIS D. WARNER and OSCAR E. ARAUJO

Abstract □ The kinetics of the rheological properties of acacia solutions prepared from two different lots of USP grade acacia were investigated with respect to preservative, temperature, and pH. A rotating viscometer was used to measure the viscosity of samples stored at 40, 50, 60, and 70° for up to 6 weeks. Solutions were prepared using benzoic acid as a preservative. Control solutions containing no preservative were also studied. An analog computer was used to analyze the viscosity and time. An Arrhenius-type relationship was established for the apparent first-order rate constants.

Keyphrases □ Acacia solutions—rheological properties □ Rheological properties, acacia solutions—kinetics □ Temperature, aging effects—acacia solutions, viscosity □ Preservative effects, acacia solutions—rheological properties

It was reported that solutions of acacia undergo a change in viscosity with time (1–4). Taft and Malm (1) found that bacterial growth lowered the viscosity of acacia solutions, while Osborne and Lee (2) reported that unpreserved acacia solutions exhibited a greater decrease in viscosity with aging than did acacia solutions preserved with 0.2% benzoic acid. Joslin and Sperandio (3) studied the effect of temperature and method of preparation on acacia solutions. They reported that the temperature of the water used to prepare the solutions affected the viscosity of the acacia solutions. They also reported that storage temperature affected the change in viscosity.

More recently, Araujo (4) studied the effects of certain preservatives on the aging characteristics of acacia solutions for a period of 1 year; he reported that acacia solutions, in a range of 10–25% by weight, behaved as Newtonian systems. He observed that the viscosity of all preserved and unpreserved acacia solutions decreased with aging. After 6 weeks at room temperature, the reduction in viscosity of the acacia solutions appeared to follow a zero-order process.

The literature contains very few kinetic studies on change of viscosity of aqueous solutions of gums. Levy and Schwarz (5) characterized the viscosity reduction of tragacanth solutions as a zero-order process, while Tobolsky (6) reported that the degradation of most linear polymers followed a first-order process.

Acacia in solution can be hydrolyzed by using sulfuric acid (7) or by heating the solution for a period of time (8, 9).

One report indicated the possibility of a relationship between viscosity and molecular weight of acacia (10). An earlier investigation indicated that acacia with a molecular weight of 280,000 may be split into fragments with molecular weights of less than 10,000 after autohydrolysis for 1 day (11). This combined information seemed to imply that there might be a change in viscosity upon hydrolysis which may be related to the change in molecular weight. Therefore, it seemed reasonable that the rate of change in viscosity might be related to the rate of hydrolysis of acacia.

If bacterial growth affects the viscosity of acacia solutions, the rate of viscosity change might also be affected by the rate of bacterial growth. Araujo (4) speculated that the rate of bacterial decomposition of acacia solutions stored at room temperature appeared to decrease after a period of 6 weeks.

Since the greatest decrease in viscosity of acacia solutions appeared to occur in the first 6 weeks (3, 4), the kinetics of the rheological properties of acacia solutions over this initial time period warranted investigation,