nant film as well as a decrease in its rate of interaction with this pharmacologically important anionic exchange resin.

SUMMARY

The present investigation has established, apparently for the first time, that the bile salt anion–cholestyramine reaction occurs by means of apparent second-order kinetics and that the reaction rates are dependent on the chemical structure of the bile salt anion participating in the interaction. Under conditions of mild agitation, it was shown that the reaction rates decrease in the following order: glycodeoxycholate > taurocholate > glycocholate, which parallels the affinity with which they bind to the resin. It is also of physiological interest that the addition of inorganic electrolyte to the system markedly depresses the rate of binding of the taurocholate and glycocholate anions to cholestyramine, which helps to explain why the resin is not as efficient as it should be *in vivo* based on the recommended dosage levels prescribed.

Both an increase in the temperature and the agitation intensity employed in the kinetic studies potentiated the rate of interaction between the glycocholate or glycodeoxycholate anion and cholestyramine. These data suggest that the binding process is most probably film diffusion controlled (27).

Additional kinetic studies are being conducted in this laboratory in order to ascertain the influence of such parameters as resin particle size and the influence of other endogenous substances on this pharmacologically important resin–bile salt interaction.

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Toxogonin: Blood Levels and Side Effects after Intramuscular Administration in Man

FREDERICK R. SIDELL and WILLIAM A. GROFF

Abstract □ Toxogonin, given intramuscularly to 10 healthy young men in doses of 2.5–10 mg./kg., produced dose-related oxime whole blood levels of 6.3–26.5 mcg./ml. and had a plasma half-time of 82.8 min. Associated side effects included tachycardia, hypertension, and a dose-independent symptom complex consisting of peroral warmth, paresthesia and hypalgesia, and a menthol taste. Some published data on the relative effectiveness of toxogonin and pralidoxime (chloride and methanesulfonate) suggest further inquiry into toxogonin's therapeutic potential may be needed.

Keyphrases □ Toxogonin, intramuscular injection—oxime blood levels □ Plasma half-life—toxogonin □ Dose relation, toxogonin—plasma blood levels □ Urinary excretion—toxogonin □ Side effects—toxogonin

Until about a decade ago the only therapy for poisoning by cholinesterase inhibitors was atropine or another anticholinergic drug plus supportive therapy. In the 1950's the oximes were developed and their usefulness as adjuncts to atropine was established (1, 2).

On the basis of therapeutic potency, relatively low toxicity in man, and other factors, the oxime generally adopted for use is pralidoxime chloride (2-PAMCl;

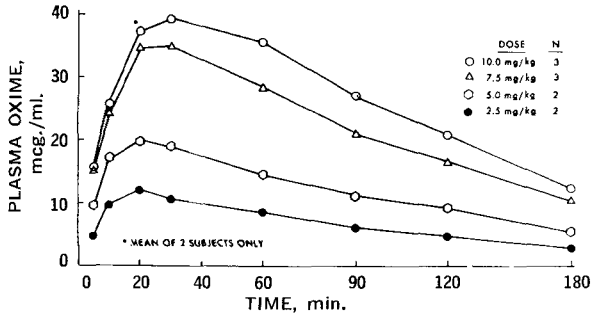

Figure 1—*Toxogonin (i.m.): plasma oxime values* versus *time.*

2-pyridine aldoxime methochloride). However, largely because of the enthusiasm of Engelhard and Erdmann (3, 4), interest has been generated in a newer oxime, toxogonin [LuH6; *N,N'*-oxydimethylene bis(pyridinium-4-aldoxime)dichloride], said to be more effective than pralidoxime chloride. It is similar structurally to TMB-4 [*N,N'*-trimethylene bis(pyridinium-4-aldoxime) dibromide], which has been shown to be more active than pralidoxime chloride therapeutically (5–8) but also more toxic in man (9, 10).

Most reports on the administration of toxogonin to humans would appear to be those of Erdmann and Engelhard. They reported that toxogonin (250 mg., i.v.) was superior to pralidoxime chloride in three cases of E-605 (parathion) poisoning (11), and they studied symptoms and blood levels of oxime in 12 healthy young male volunteers who were given 250 mg. of toxogonin intramuscularly in a 25% solution (12). The purposes of this study were: to extend these latter observations over a wider dose range, to study the blood levels and excretion patterns of this oxime, and to determine what, if any, undesirable side effects would be produced.

SUBJECTS AND METHODS

The subjects were U. S. Army enlisted men, ages 18 to 24 years (average 21 years), who volunteered for this study. Each had a complete medical evaluation including a physical examination, chest X-ray, electrocardiogram, and laboratory tests [hematocrit, total and differential white blood cell count, urinalysis, including microscopic examination, blood urea nitrogen (BUN), serum glutamic oxaloacetic transaminase (SGOT), alkaline phosphatase, bilirubin, serum creatinine, and red blood cell and plasma cholinesterase], and they were found to be free from any abnormality before being accepted into the study. They were told that they would take part in a study of a new and possibly very effective antidote for nerve agent poisoning; the test procedures were described to them, including the necessity of multiple venipunctures. They were told that there might possibly be some symptoms, but these were left unspecified. The only reward for participation was an extra day off.

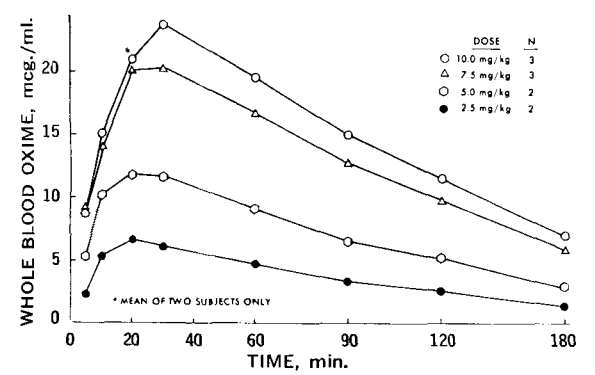

Figure 2—*Toxogonin (i.m.): whole blood oxime levels* versus *time.*

The volunteers slept in the ward the evening before the test. Fluid intake was maintained at a high level prior to and throughout the test. During the hour before the drug was administered, three measurements were recorded of supine blood pressure, heart rate, and pupil size. After appropriate control urine and blood specimens had been taken, the subjects ate a light breakfast.

Except to void, the subjects remained in bed during the first 3 hr. of the test; for the remainder of the 24-hr. test period, they were allowed to be up and around the ward. Blood pressure and heart rate were measured every 15 min. for 2 hr. after administration of the drug. Blood samples were taken at 5, 10, 20, 30, 60, 90, 120, and 180 min. after drug. The subjects were asked to void every 30 min. for 3 hr.; for the next 21 hr., all urine was collected, but no attempt was made to follow a definite schedule.

Whole blood, plasma, and urine were analyzed for oxime content by the method described by Groff and Ellin (13). Blood and plasma were analyzed within minutes of collection; the urine was refrigerated and analyzed several days later. Preliminary studies performed by adding a known amount of toxogonin to urine and making daily determinations of toxogonin content showed that there was insignificant (under 5%) deterioration of the oxime over a 1-week period. The oxime content of red blood cells was also measured and will be the subject of a separate report.

Other blood and urine studies (hematocrit, total and differential white blood cell count, SGOT, alkaline phosphatase, BUN, and urinalysis) were performed by routine analytical methods on samples taken at 24 hr. and 7 days. Red cell and plasma cholinesterase of the three subjects receiving the highest dose was measured in samples taken at 20 min., 30 min. (times of peak oxime levels), and 3 hr. after administration of the oxime by methods previously described (14).

The subjects' average weight was 76 kg. (range 56–93 kg.). The volume of injection ranged from 0.64 to 3.1 ml., and the injection was given into the deltoid muscle with a No. 23 needle. Doses were 2.5 mg./kg. (two subjects), 5.0 mg./kg. (two subjects), 7.5 mg./kg. (three subjects), and 10.0 mg./kg. (three subjects). All doses and blood levels are expressed as the salt of the oxime.

Toxogonin was purchased from E. Merck, Darmstadt, West Germany. Recent studies on the sample used showed that it was over 98% pure by UV spectroanalysis and its LD_{50} [calculated by the method of Bliss (15)] by the intraperitoneal route in 170–200-g. female rats was 109.6 (95% CL: 102.5–117.2) mg./kg. It was dissolved in distilled water to a concentration of 274 mg./ml. and filtered through a Seitz filter. Bacteriological analysis showed it to be sterile.

RESULTS

A. Blood Levels of Oxime—Serial mean plasma and whole blood levels for toxogonin for each dose group are shown in Figs. 1 and 2. Peak blood levels were reached at 20 min. in the two lower dose groups and at 30 min. in the higher dose groups. All groups had plasma values above 5 mcg./ml. by 5 min., and all but the group that received 2.5 mg./kg. maintained a plasma level of about 4 mcg./ml. for over 3 hr. (Because of technical difficulties the analysis of the blood taken at 20 min from one subject who received 10 mg./kg. was not measured; the point is the mean for the other two subjects. The data from this subject were also excluded from dose–response calculations.)

The dose–response relationship, showing the highest plasma value for each subject, is shown in Fig. 3.

The disappearance from plasma of many drugs follows first-order kinetics, $C = C_0e^{-kt}$, where C = concentration at time t; C_0 = apparent concentration at zero time, *i.e.*, the y-intercept; and k = the velocity constant characterizing drug-elimination rate from the blood stream. The half-life ($t_{1/2}$) can be determined most simply by plotting blood levels on a logarithmic scale against time on a linear scale and, if the terminal segment of the plot is linear, calculating from its slope the half-life ($t_{1/2}$) and k value (the rate constant characterizing elimination from the blood stream).

Using the method of least squares, the constants log C_0 and k for the equation

$$\log C = \log C_0 - \frac{kt}{2.303} \qquad \text{(Eq. 1)}$$

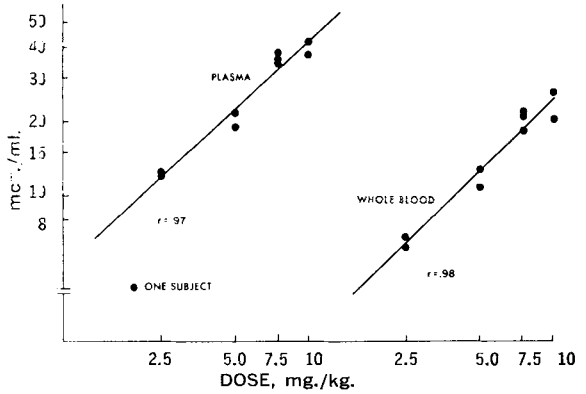

Figure 3—*Toxogonin (i.m.): peak blood levels* versus *dose.*

were computed from each subject's last 4–6 plasma level values. In each case the coefficient of correlation for the fit of the points to the line was greater than 0.99.

In Fig. 4 the data from four typical subjects are plotted in this manner. The average $t_{1/2}$ was 82.8 min. [the mean values by dose groups were 79.4 min. (2.5 mg./kg.), 85.6 min. (5.0 mg./kg.), 82.7 min. (7.5 mg./kg.), and 84.0 min. (10.0 mg./kg.)]; and the average k value was 0.0083 min.$^{-1}$ (by dose groups in min.$^{-1}$: 0.0087 for 2.5 mg./kg., 0.0081 for 5.0 mg./kg., 0.0082 for 7.5 mg./kg., and 0.0083 for 10 mg./kg.).

B. Urinary Excretion—Of the total dose, 84% was excreted into the urine unchanged during the 24-hr. collection period; of this amount, 76% (or 64% of the dose) was excreted in the first 3 hr. (Because of technical problems, data from one subject at the 10-mg./kg. dose could not be used in the urinary analysis.) The percentage of the amount not excreted:

$$\frac{(\text{total amount excreted} - \text{amount excreted by time } t) \times 100}{\text{total amount excreted}}$$

is plotted on a logarithmic scale against time, t, in Fig. 5. Times for 50% excretion for each dose group are 73.0 min. (2.5 mg./kg.), 72.0 min. (5.0 mg./kg.), 110.4 min. (7.5 mg./kg.), and 107.4 min. (10 mg./kg.) and are close to the biological half-life as measured by loss from plasma. This suggests that a large part of the oxime is eliminated rather rapidly by renal mechanisms.

C. Blood Pressure and Heart Rate—All subjects showed a mild to moderate transient increase in blood pressure and heart rate (Figs. 6 and 7). The small number of subjects in each dose group makes exact quantitation of such variables unreliable, but both the systolic and diastolic blood pressures were moderately elevated (11–17 mm. Hg), reaching peak values at about 30 min. after drug administration. The heart rate change was more dose related with an average maximal increase of 8 b.p.m. for 2.5 mg./kg., 15 b.p.m. for 5.0 mg./kg., and 32 b.p.m. for 10 mg./kg. In general the heart rate increased markedly a few minutes after drug administration (data for the lower doses are not shown because heart rates were not measured earlier than 15 min. for the lower dose subjects) and remained elevated for most of the 2-hr. observation period.

D. Other Blood and Urine Studies—No changes were noted in any of the routine blood or urine tests at 24 hr. or 7 days in any subject, nor were there changes in the red blood cell or plasma cholinesterase in the three subjects in whom it was measured.

E. Symptoms—Pain at the site of injection was quite noticeable, particularly with the larger volumes used for the higher doses, and most subjects compared it in intensity to the pain from an injection of plague or Asian influenza vaccine. Within a few minutes the pain seemed to diminish greatly, and by 15 min. most subjects said they did not notice it. A slight residual tenderness of the muscle to percussion was present in all subjects 24 hr. later.

A peculiar symptom complex developed in many subjects. This typically consisted of a generalized warmth, a "hot feeling" over the upper part of the body, within several minutes after the injection. At 5–15 min., this became localized in the face, particularly around the mouth, where it was associated with a "tight feeling" of the skin and muscles of the lower face or forehead and a "numbness" of the circumoral area. Nine subjects (all but one at the highest dose) noted a hot feeling in their throats within 1–3 min. after injection, and three

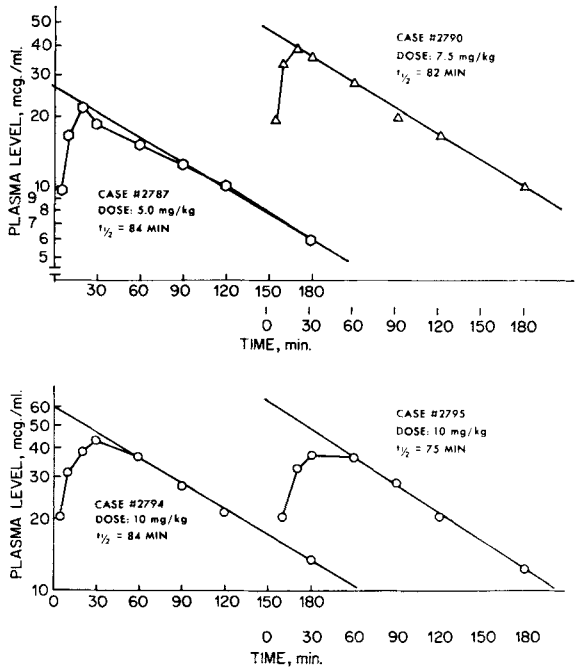

Figure 4—*Toxogonin (i.m.): plasma levels and half-times in four subjects.*

of these men (2.5, 5.0, and 10.0 mg./kg.) also spontaneously identified this as a "menthol" taste. One subject at 2.5 mg./kg., one at 5.0 mg./kg., and one at 10.0 mg./kg. reported a numbness in or around the mouth, together with a definite hypalgesia to pinprick in this area including the anterior tongue and gums (two subjects), which lasted from 3 min. to 6 hr. after injection. No depression of the corneal reflex or muscular weakness in the face was observed. When present, the area of involvement was sharply demarcated. In one, it was from midforehead to just under the chin; in another, it had a definite 5.08–7.62-cm. (2–3-in.) radius around the mouth. Most reported a flushing sensation, but one subject said he felt very hot with

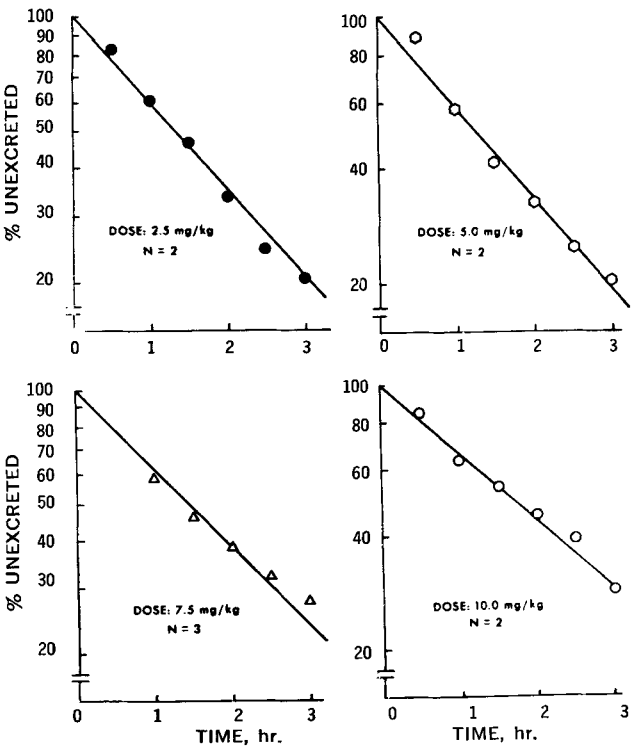

Figure 5—*Urinary excretion of toxogonin: percent unexcreted* versus *time.*

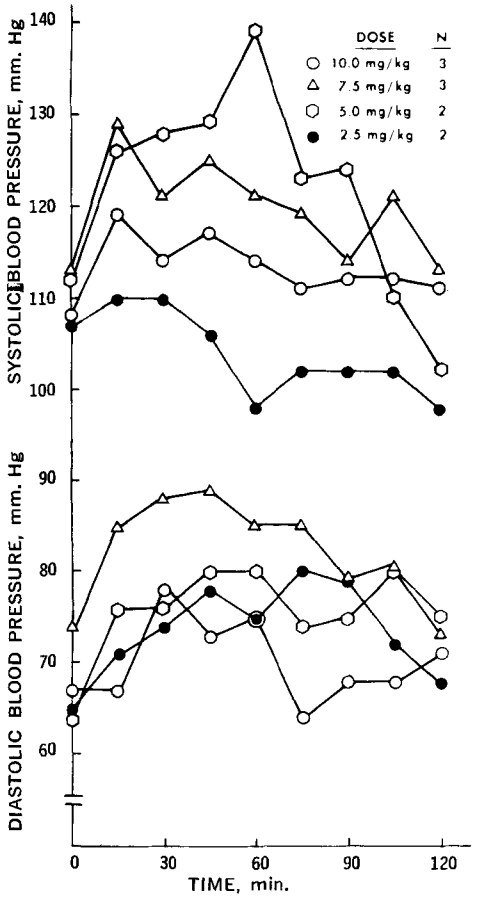

Figure 6—*Toxogonin (i.m.): blood pressure changes* versus *time.*

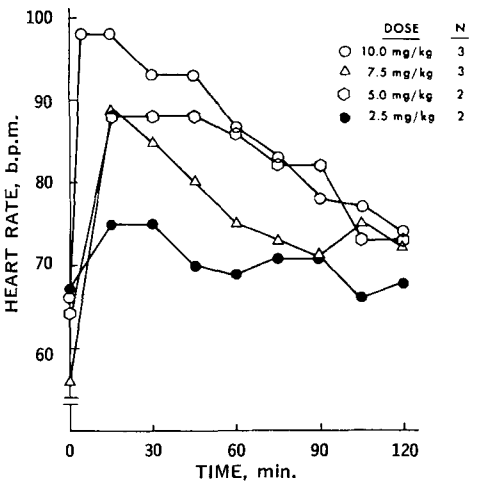

Figure 7—*Toxogonin (i.m.): heart rate changes* versus *time.*

cool flashes. The skin of several subjects was very warm to the touch and appeared slightly pink. Several noted "pins and needles" sensations in their arms and fingers, not associated with hyperventilation. All three subjects at the highest dose reported their eyes "felt like baseballs" with heaviness in the eyeballs or a feeling that "they're enlarging." Only one subject (7.5 mg./kg.) reported nausea and this was very transient. A dry mouth was a unanimous symptom of all subjects in the highest dose groups.

Except for mild paresthesia and, in two cases, mild hypalgesia, all symptoms had subsided by 1–2 hr.

DISCUSSION

In a study of blood levels and urinary excretion of toxogonin after intramuscular administration to 12 healthy young men, Erdmann *et al.* (12) gave an average dose of 3 mg./kg. They noted a peak whole blood oxime level of about 6 mcg./ml. at 30 min., found 52% of the dose in the urine by 2 hr., and reported that their subjects complained of "heat and tension" in the facial area and a menthol-taste sensation in the nasopharynx. In the study reported here, the two subjects who received 2.5 mg./kg. had a peak whole blood level of oxime of 6.6 mcg./ml. at 20 min., and the average excretion for nine subjects was 53% of the dose by 2 hr. The subjects reported the same symptoms and, in addition, had transient neurological findings.

Calesnick *et al.* (10) found that an intramuscular dose of 15 mg./kg. of pralidoxime chloride produced whole blood oxime levels of 8.9 mcg./ml. at 30 min. and 5.7 at 2 hr., levels exceeded by 5.0 mg./kg. of toxogonin at these times, and a dose of 30 mg./kg. produced whole blood levels (14.5 mcg./ml. and 10.4 mcg./ml. at similar times) below those produced by 7.5 mg./kg. of toxogonin. Thus within the ranges studied, three to four times more pralidoxime chloride than toxogonin is needed for a similar blood level.

Pralidoxime chloride in intravenous or intramuscular doses of less than 15 mg./kg. does not elevate the blood pressure in normal recumbant subjects (10, 16, 17). This sympathomimetic property of toxogonin may be of additional therapeutic benefit for the severely poisoned individual.

The cause of the peculiar symptom complex produced by toxogonin is not clear. Because of their "onion skin" pattern, the sensory disturbances of the face resemble those caused by a lesion of the nucleus or descending fibers of the sensory division of the trigeminal nerve in the medulla or upper spinal cord (18), although the production of such a lesion by a drug would be quite rare.

With high doses of pralidoxime chloride (15–30 mg./kg., i.v.), diplopia has been noted[1] (16). The authors also have observed this with pralidoxime chloride (20–30 mg./kg., i.v.) associated with demonstrable weakness of the muscles supplied by the third, fourth, and sixth cranial nerves. It is possible that this side effect is also central in origin.

The usefulness of toxogonin in clinical therapy is unknown, although, as noted, Erdmann and Clarmann (11) felt that toxogonin was more beneficial than pralidoxime.

The relationship of oxime blood levels to therapeutic efficacy is also unknown. Erdmann *et al.* (12), on the basis of *in vitro* studies (3) showing toxogonin to be 30 times more effective than pralidoxime in reactivating organophosphate-inhibited cholinesterase, suggest that the level of 4 mcg./ml., reported to be an effective *in vivo* level of the methanesulfonate salt of pralidoxime (P2S) by Sundwall (19), would be more than adequate. Using identical *in vitro* techniques in a comparative study, they also found that blood concentrations of 0.1–0.2 mcg./ml. reactivated cholinesterase by the same amount as concentrations of 3 mcg./ml. of pralidoxime (a concentration very close to that suggested by Sundwall from *in vivo* work).

However, several studies in which toxogonin (or the chemically similar TMB-4) has been compared with pralidoxime (or P2S, the methanesulfonate salt of pralidoxime) indicate that perhaps this advantage is not quite so great (20, 21).

Both toxogonin and TMB-4 have two oxime groups per molecule and, therefore, on an equimolar basis might be expected to have twice the efficacy of a compound having a single oxime group, such as pralidoxime. (On the other hand, yoked oxime groups might be less effective than the same number of single oxime units.) It has been shown that toxogonin and TMB-4 are roughly equipotent in treating poisoned mice (22), in reactivating blood (mouse) or brain (mouse and rat) cholinesterase inhibited (*in vivo*) by an organophosphate compound (diethyl *p*-nitrophenyl phosphate)[2] (23), or in antagonizing the effects of tabun on the isolated rat phrenic nerve preparation (20).

O'Leary *et al.* (6) showed that 10 mg./kg. (28 μmole/kg.) of TMB-4 with atropine raised the LD_{50} of sarin in rabbits by a factor of 170, whereas 10 mg./kg. (58 μmole/kg.) of pralidoxime chloride raised the LD_{50} by 90, and the same dose of P2S (41 μmole/kg.) raised it by 35. This indicates that, for equimolar amounts, TMB-4 is roughly 3.9 times more effective than pralidoxime chloride and 7.5 times more effective than P2S. Fleisher *et al.* (5) showed that equimolar doses

[1] B. Calesnick, personal communication.
[2] Paraoxon.

of TMB-4 were from 1.4–7.0 times more effective than pralidoxime in raising the LD_{50} of difluorophosphate, sarin, tabun, and tetraethylpyrophosphate in rats.

This latter group of investigators (21) also showed that, in equimolar doses, toxogonin and atropine approximately doubled the LD_{50} of sarin in rats (1.6×) and guinea pigs (2.2×) over that in animals treated with pralidoxime chloride and atropine. When given on an equiweight basis, the two oximes had nearly the same therapeutic activity. LD_{50}'s done by probit analysis [method of Bliss (15)] from data in Table II of the Wolthuis and Cohen report (20) suggest that toxogonin is about 1.7 times more effective therapeutically than P2S against tabun in rats (also receiving atropine) or 2.6 times more effective on an equimolar basis. Heilbronn and Tolagen (22) also showed that in atropinized mice, toxogonin is 4.6 times more effective against sarin and 9.5 times more effective against tabun than P2S when the dose of each oxime is 20% of its LD_{50}. On an equimolar basis, these ratios are approximately doubled to 7.1 and 14.5.

Overall, it would appear that toxogonin may be more effective therapeutically than pralidoxime chloride or P2S, although in two of the three studies where comparisons were made, the therapeutic difference was not marked.

The data in these reports also indicate that in each case, except the guinea pig experiments, the oxime that is more therapeutically potent also has a lower LD_{50} (is more toxic). However, the "therapeutic margin" (potency ratio/LD_{50} ratio) indicates that, in most cases, effectiveness is gained in greater proportion than toxicity, at least when toxogonin and pralidoxime chloride (or P2S) are compared directly.

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