

QUANTITATIVE STUDY OF THE ACTION OF IMBRETIL AND ITS MODIFICATION IN MAN

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THE PRESENT study was undertaken to determine as precisely as possible the duration of paralysis produced by Imbretil, hexamethylene 1,6 bis-carbaminoyl choline bromide, on the diaphragm, the external oblique abdominis and the flexor muscles of the forearm in man. The efficacy of various "antagonists" in relieving the neuromuscular block of these three groups of muscles was also studied.

MATERIALS AND METHODS

Twenty patients of both sexes ranging in weight from 45.5 to 72 kg. and scheduled to have minor surgical procedures were studied. Preanesthetic medication consisted of secobarbital, meperidine and scopolamine in average therapeutic doses. Light anesthesia was induced with thiopental or cyclopropane and maintained in as uniform a level as possible with nitrous oxide (60–70 per cent) and oxygen (30–40 per cent) and intermittent or slow continuous intravenous infusion of 0.2 per cent thiopental solution. An endotracheal tube was inserted with the aid of surface anesthesia (2 per cent lidocaine solution) of the pharynx, larynx and trachea. No relaxant was used for endotracheal intubation. The endotracheal tube was connected to a research model volume ventilator ("Snorky") through a nonrebreathing valve. Artificial respiration was so adjusted that the end-tidal P_{CO_2} as measured with a Beckman infrared CO_2 analyzer was maintained at or near normal control values. Arterial blood samples were obtained every 15–30 minutes for the determinations of pH, Pa_{CO_2} , buffer base and O_2 saturation. The Pa_{CO_2} agreed closely with the end-tidal P_{CO_2} in that there was a mean difference of 2 mm. Hg. The arterial oxygen

saturation was usually 96–98 per cent and was never below 93 per cent.

A pneumotachograph was placed next to the inspiratory arm of the nonrebreathing valve and its signal was recorded on a multichannel cathode ray oscillograph. The output of the pneumotachograph was integrated electronically. The integrated pneumotachogram was used as an index of inspiratory tidal volume. The electrical activities of the diaphragm and the external oblique abdominis (electromyograph, EMG) were recorded with two sets of bipolar electrodes and appropriate amplifying systems. Twitch tension of the forearm flexors in response to ulnar nerve stimulation was detected with a strain gauge and recorded on the oscillograph. Stimulating currents were derived from a Grass stimulator (Model S40R) through an isolation unit and applied to the nerve near the medial epicondyle of the elbow through a needle electrode. Monophasic square-wave pulses with an intensity varying from 8 to 15 volts and a duration of 0.1 to 4 milliseconds were delivered at a frequency of 1 shock per 4 seconds. The parameters were kept constant throughout any one experiment.

After 5–10 minutes of observation and stabilization Imbretil in doses of 15 to 91 μ g./kg. (1–6 mg.) was injected intravenously. The onset of paralysis was judged by the disappearance of diaphragmatic and abdominal EMG and forearm flexor twitch. In seven subjects the natural course of recovery of muscle function was observed. In thirteen subjects, one of several "antagonists" was injected intravenously at the time when there was a minimal recovery of the forearm flexor twitch and the beginning of a spontaneous respiratory effort. The subsequent course of recovery was observed. These agents and their doses were: neostigmine methylsulfate, 2 mg.; edrophonium chloride, 10 mg.; B.W. Drug No. 49–204, 3–4 mg./kg.;¹ thiamine

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chloride, 7.5–10 mg./kg.² and pyridine-2-aldoxime methiodide (2-PAM), 15–20 mg./kg.³ Atropine sulfate (1 mg.) was administered prior to the injection of neostigmine and edrophonium.

In a few instances after apparent complete recovery of muscle function the study was repeated with small divided doses of Imbretil (0.25–0.5 mg.) and the same "antagonist." No subject received more than one "antagonist."

In four additional subjects Imbretil was administered during a surgical procedure and studies carried out after the completion of operation when there was residual paralysis and hypoventilation. Not all of the parameters mentioned above were measured under these circumstances.

RESULTS

During light thiopental, nitrous oxide-oxygen anesthesia and prior to the administration of Imbretil, the forearm flexor twitch tension was steady and showed only insignificant variations. The diaphragmatic EMG bursts were phasic with a certain amount of background activity. These inspiratory bursts were both in and out of phase with the ventilator. The external oblique EMG showed continuous activity with increased discharge during expiration. The tidal volume of the subjects' spontaneous respiratory effort, measured with the ventilator turned off for the duration of one or two breaths, was relatively constant.

Effect of Imbretil on the Twitch of the Flexor Muscles of the Forearm. Enough Imbretil (32–91 μ g./kg., 2–6 mg.) was injected intravenously to produce complete muscular paralysis within 2–3 minutes as indicated by the disappearance of the forearm flexor twitch. Complete paralysis lasted from 20 to 45 minutes. One subject received only 15 μ g./kg. (1 mg.) of Imbretil and had only partial paralysis. The twitch tension was reduced to 3 per cent of the control value. Recovery began in 10 minutes. The correlation coefficient between the dose of Imbretil and the duration of complete paralysis was 0.553.

The time required for complete recovery of twitch tension could be measured only in the seven subjects who received no "antagonist." In five of these full recovery took place from

30 to 60 minutes after the injection of 47–71 μ g./kg. (3–4 mg.) of Imbretil. In the remaining two who received 88 and 91 μ g./kg. (4 and 6 mg.) of Imbretil respectively, recovery was not complete after 70 minutes in one case and 90 minutes in the other.

Effect of Imbretil on the Activity of the Diaphragm and the External Oblique Abdominis. Apnea was produced within 2–3 minutes after the injection of Imbretil. The phasic discharges of the diaphragm were abolished at the same time or 1–2 minutes afterward. The electrical activity of the external oblique abdominis usually stopped at the same time as the diaphragm but occasionally persisted longer for various lengths of time (up to 9 minutes) depending upon the dose of Imbretil. During the apneic period the diaphragm remained electrically "silent" except in a few instances where traces of random discharge of extremely low amplitude were observed. The electrical "silence" of the diaphragm lasted from 14 to 33 minutes, after which phasic activity was discernible both on the monitoring screen and from the audio monitoring system. The correlation coefficient between the dose of Imbretil and the duration of diaphragmatic "silence" was 0.77. Coincident with or a few minutes after the return of phasic diaphragmatic electrical activity, spontaneous respiration began and the tidal volume increased progressively with time.

In the subject who received only 15 μ g./kg. (1 mg.) of Imbretil, diaphragmatic and abdominal muscular activity was not abolished.

Effect of "Antagonists" on the Action of Imbretil. In twenty instances (17 subjects) one of the "antagonists" was administered at

TABLE I
EFFECTS OF "ANTAGONISTS" ON NEUROMUSCULAR BLOCK PRODUCED BY IMBRETIL (AS MEASURED BY FOREARM FLEXOR TWITCH TENSION AND SPONTANEOUS TIDAL VOLUME)

Drug	Dose	No. of Tests	Effect		
			Antagonist	Potentiator	No Effect
Neostigmine	2 mg.	6	3	—	3
Edrophonium	10 mg.	1	—	—	1
Thiamine	7.5–10 mg./kg.	8	5	2	1
B.W. 49-204	3–4 mg./kg.	2	—	2	—
2-PAM	15–20 mg./kg.	3	—	2	1

the beginning of the recovery of forearm flexor twitch. The results are presented in the table. Antagonism was considered effective if the forearm flexor twitch tension, spontaneous respiratory tidal volume, and the diaphragmatic EMG showed an increase or an accelerated rate of recovery within 2–3 minutes after the injection of these agents. If the twitch tension and the spontaneous respiratory tidal volume became depressed following the injection, potentiation of Imbretil action was thought to have occurred. A third possibility was also observed—no effect at all.

Under the conditions of this study, neostigmine did not relieve the block produced by Imbretil in clearly predictable fashion. In the three instances where the dose of Imbretil was moderate, *i.e.*, 44–65 $\mu\text{g./kg.}$ (3–4 mg.), the beginning of recovery of the forearm flexor twitch and spontaneous respiratory effort occurred within 30 minutes. Neostigmine, administered shortly afterwards (35–40 minutes after Imbretil) did not seem to have any fur-

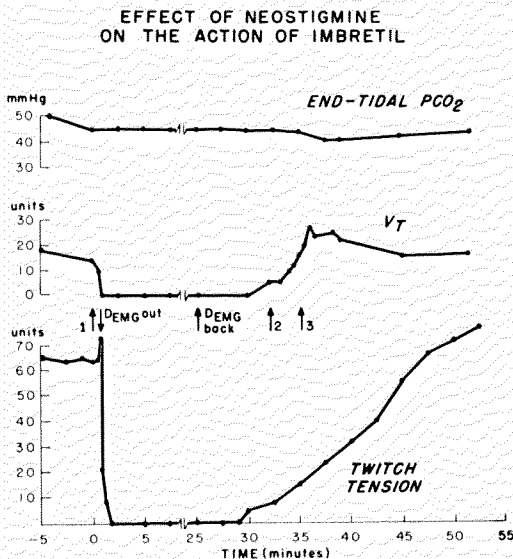


FIG. 1. Female, 68 kg. Spontaneous respiratory tidal volume (V_T) is indicated as arbitrary units measured from the integrated pneumotachogram. At arrow 1, Imbretil, 44 $\mu\text{g./kg.}$ (3 mg.) was injected intravenously. Both the forearm flexors and diaphragm became paralyzed in 1 minute. At arrow 2, atropine, 1 mg., intravenously and at arrow 3, neostigmine, 2 mg., intravenously. Neostigmine did not appear to alter the rate of recovery of V_T and twitch tension. End-tidal P_{CO_2} was kept constant with artificial respiration.

EFFECT OF NEOSTIGMINE ON THE ACTION OF IMBRETIL (Late antagonism)

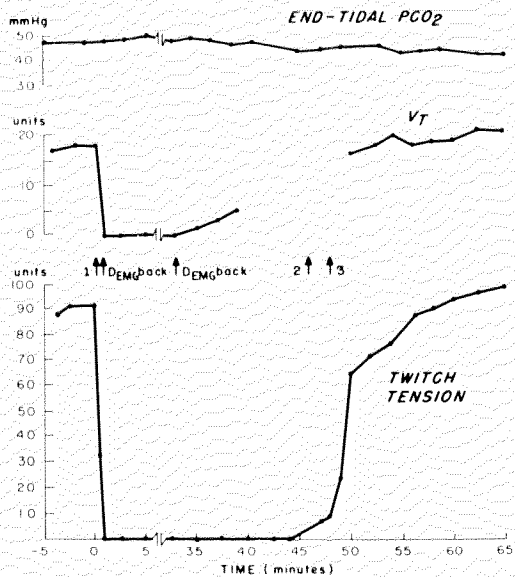


FIG. 2. Female, 60 kg. At arrow 1, Imbretil, 83 $\mu\text{g./kg.}$ was injected intravenously. Apnea and paralysis of forearm flexors occurred in 1 minute. At arrow 2, atropine, 1 mg., and at arrow 3, neostigmine, 2 mg., intravenously. Note immediate increase in twitch tension which returned to control value in 5 minutes. Break in tidal volume curve represents temporary breakdown of recording apparatus.

ther influence on the rate of recovery. The result from one study is shown in figure 1. In three subjects the dose of Imbretil was relatively large (83–90 $\mu\text{g./kg.}$, 5–6 mg.) and the duration of paralysis was longer (40–45 minutes). Neostigmine given under these circumstances (approximately 50 minutes after Imbretil) was followed by an accelerated rate of recovery of twitch tension and spontaneous respiratory effort. These functions reached the control values within 5 minutes after the injection of neostigmine (fig. 2).

Edrophonium was tried once in a dose of 10 mg. and produced no apparent effect.

The results obtained with thiamine differed in different trials. In five subjects, thiamine relieved the block when 10 mg./kg. was injected within 40 minutes after the administration of Imbretil and when the spontaneous respiratory effort and the forearm flexor twitch had begun their recovery. Figure 3 illustrates

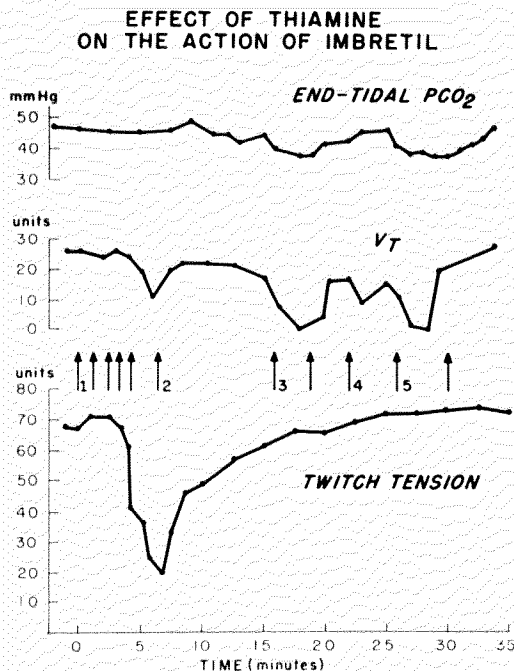


FIG. 3. Female, 63 kg. Fifty minutes prior to zero time Imbretil, 32 $\mu\text{g./kg.}$ (2 mg.) was injected intravenously. Twelve minutes prior to zero time intravenous injection of thiamine, 10 mg./kg., effectively antagonized the action of Imbretil. At arrows 1, 0.25 mg. each of Imbretil (total, 1.25 mg., 20 $\mu\text{g./kg.}$) were injected intravenously. At arrow 2, thiamine, 10 mg./kg., again antagonized the action of Imbretil. At arrows 3 and 5, passive hyperventilation reduced end-tidal P_{CO_2} , resulting in apnea. At arrow 4, thiopental, 150 mg., reduced spontaneous tidal volume (V_T). Twitch tension was not affected by hyperventilation and thiopental.

the result in one of these cases. In two instances thiamine caused a transient depression of spontaneous respiratory tidal volume and twitch tension. One of these subjects was studied after operation. He had common bile duct obstruction and icterus. Imbretil, 55 $\mu\text{g./kg.}$ (4 mg.) was administered during cholecystectomy and common duct exploration. At the completion of operation the spontaneous respiratory tidal volume was inadequate and thiamine (10 mg./kg.) was administered (110 minutes after Imbretil). The result is shown in figure 4. Artificial respiration was continued for 30 minutes until spontaneous respiratory exchange was considered adequate.

B. W. Drug No. 49-204, in doses of 3-4 mg./kg., was tried in two instances as recovery began and caused a transient depression of

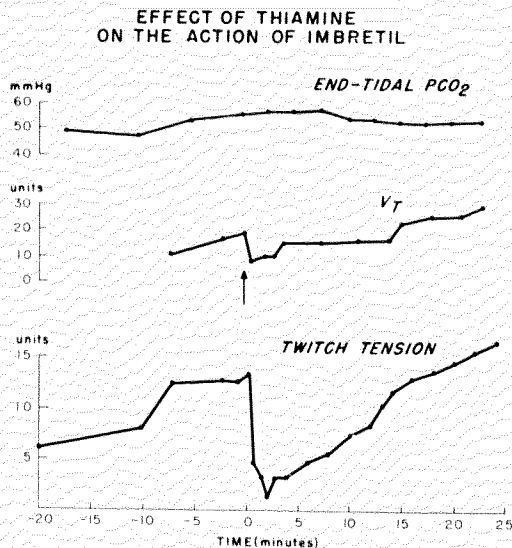


FIG. 4. Male, 73 kg. Imbretil, 55 $\mu\text{g./kg.}$ (4 mg.) was given 110 minutes prior to zero time during cholecystectomy and common duct exploration. At arrow thiamine, 10 mg./kg., reduced the spontaneous tidal volume (V_T) and the forearm flexor twitch tension.

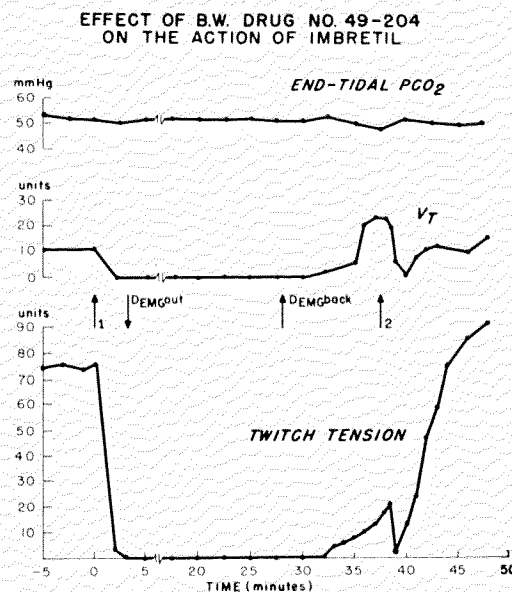


FIG. 5. Female, 61.5 kg. At arrow 1, Imbretil 49 $\mu\text{g./kg.}$ (3 mg.). Apnea and paralysis of forearm flexors occurred in 2 minutes. Phasic diaphragmatic EMG disappeared in 3 minutes. At arrow 2, B.W. Drug No. 49-204, 3 mg./kg., caused transient potentiation of the action of Imbretil, decrease in twitch tension, and spontaneous tidal volume (V_T).

forearm flexor twitch tension and spontaneous respiratory tidal volume (fig. 5). Pyridine-2-aldoxime methiodide (15–20 mg./kg.) also produced a transient depression of the twitch tension in two instances and no effect in one. However, while the twitch tension was depressed, there was an increase in the EMG activity of the diaphragm with this drug.

DISCUSSION

The use of muscle twitch tension as measurement of the action of neuromuscular blocking agents and its modification is a standard laboratory procedure. Its application in human studies has not been frequent although data obtained in this manner can be of significance in the understanding of the action of relaxants in man.

Most clinical studies of relaxants have been conducted using minute or tidal ventilatory volume alone as the critical measurements. Since the neural control of respiration during anesthesia with controlled respiration may be altered in an unpredictable manner, ventilatory volume cannot be utilized as the sole index of neuromuscular blockade. For example, it has been shown that diaphragmatic EMG and spontaneous respiration can be abolished during general anesthesia by increasing the ventilatory volume and reducing the end-tidal P_{CO_2} to levels 10 mm. Hg below the control values.⁴ It is also obvious that deepening of anesthesia can decrease the ventilatory effort. The effect of these factors on ventilation is shown in figure 3. After there was improvement of spontaneous respiratory tidal volume with the administration of thiamine, hyperventilation produced a fall of end-tidal P_{CO_2} from 44 to 38 mm. of mercury. This change was accompanied by the disappearance of phasic diaphragmatic activity and apnea. Administration of 150 mg. of thiopental also reduced the EMG activity and spontaneous tidal volume with an increase of end-tidal P_{CO_2} of 3 mm. of mercury. Both these maneuvers did not significantly alter the forearm flexor twitch tension.

From the foregoing, it is clear that if the spontaneous ventilatory volume is to be used as an index of the action of a relaxant, carbon dioxide tension in the blood should be kept at or slightly above the control level and the

depth of anesthesia as constant as possible. It is only under these conditions that the central respiratory drive can be assumed to be constant and changes in neuromuscular transmission in respiratory muscles can be studied with some degree of confidence. In this study reliance was placed on the monitoring and control of end-tidal P_{CO_2} . The depth of anesthesia was kept relatively constant by the choice of anesthetic agents and clinical signs without the aid of the electroencephalograph.

The use of artificial ventilation with a controlled level of end-tidal P_{CO_2} did not interfere with the measurement of respiratory muscular activity and spontaneous tidal volume. The most sensitive means of detecting respiratory muscular activity appears to be the EMG of these muscles. Artificial ventilation does not have to be interrupted for the observation of diaphragmatic EMG. However, the measurement of the "work" accomplished by these muscles, *i.e.*, the respiratory tidal volume, is carried out by stopping the ventilator for one or two breaths. Interruption of artificial ventilation for this duration did not alter the end-tidal P_{CO_2} significantly.

The duration of complete paralysis of the forearm flexors could not be correlated with the dose of Imbretil administered although the duration of partial paralysis was prolonged after larger doses. This is not surprising since there are probably many factors besides body weight which can influence the duration of action of a relaxant. Therefore, the dose schedule in respect to body weight and contemplated duration of surgical procedures as suggested by Reis⁵ can be used only as a rough guide.

The question of antagonism against Imbretil was not answered clearly in this study. The difficulty in study lies in the fact that the action of Imbretil is prolonged and the effect of repeated administration is cumulative. It is believed that these characteristics of the drug prevent using a subject as his own control for the study of "antagonists" since repeated injections of substantial doses of Imbretil are impractical. The recovery rate of muscular function after the administration of an "antagonist" could be compared only with that observed in other subjects in whom no "antagonist" was given. Because of differences in

the dose of Imbretil and individual variations this method of comparison is at best a gross estimation unless a clear cut marked change is observed. These data suggest that neostigmine may be effective as an antagonist only when the duration of paralysis is relatively long (approximately 1 hour or more) and the dose of Imbretil large (80 $\mu\text{g./kg.}$ or more). Contrariwise, thiamine is an antagonist only when given relatively early (less than 40 minutes) after Imbretil and it may potentiate the action of the relaxant when used after prolonged paralysis.

The results obtained with B. W. Drug No. 49-204 are surprising in view of the fact that this agent is an effective antagonist against decamethonium¹ and was found to antagonize Imbretil in the cat.⁶ This illustrates again the importance of species difference and emphasizes the need of ultimate evaluation of relaxant action in human subjects.

Pyridine-2-aldoxime methiodide is an effective antagonist against *d*-tubocurarine in the cat.³ Against Imbretil in man, it caused transient potentiation of the forearm muscle paralysis in two out of three instances. It is interesting that at the same time it increased respiratory muscular activity. The mechanism for this finding is not clear at the present. One plausible explanation is that 2-PAM may act as a central stimulant. Its potentiating effect at the neuromuscular junction may be offset by the increased central respiratory drive.

The data presented in this study are not such as to justify discussion of the mechanism of action of Imbretil nor the basis of its modification by the various agents tested.

SUMMARY

The action of Imbretil (hexamethylene 1,6 bis-carbaminoyl choline bromide), a muscle relaxant, was studied in 24 anesthetized subjects with forearm flexor twitch tension, diaphragmatic and abdominal electromyography and spontaneous respiratory tidal volumes as indices of neuromuscular block. The experi-

ments were made under controlled conditions whereby the end-tidal P_{CO_2} and depth of anesthesia were kept relatively constant.

Under the conditions of this study, Imbretil in doses of 32-91 $\mu\text{g./kg.}$ (2-6 mg.) produced apnea for 14-33 minutes and complete paralysis of the forearm flexors for 20-45 minutes. There was no statistical correlation between the dose of Imbretil and the duration of complete paralysis except that with larger doses (approximately 90 $\mu\text{g./kg.}$) the duration of partial paralysis was prolonged.

In 17 subjects the effect of various "antagonists" on the action of Imbretil was studied during the recovery phase. Neostigmine appeared to be effective as an antagonist when administered approximately an hour or more after Imbretil. Thiamine antagonized the action of Imbretil when given less than 40 minutes after Imbretil. After prolonged Imbretil paralysis thiamine caused a transient depression of muscular function. B. W. Drug No. 49-204, an antagonist against decamethonium, potentiated the action of Imbretil.

The importance of the measurement of ventilatory function under controlled conditions in the study of the action of relaxants and their "antagonists" was discussed.

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