THE ANTIVIRAL EFFECT OF SOME DERIVATIVES OF TILORONE HCl ON TBE IN WHITE MICE

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From the Institute of Virology, University of Vienna (Director: Prof. Dr. Ch. Kunz)

THE ANTIVIRAL EFFECT OF SOME DERIVATIVES OF TILORONE HCL ON TBE IN WHITE MICE [1,2]

HANNS HOFMANN AND CHRISTIAN KUNZ with technical assistance from H. DIPPE

With 1 diagram. Presented on 13 August 1973

Abstract

Four derivatives of Tilorone HCl were tested against TBE in mice and compared with Tilorone HCl. All substances induced interferon and thus protected mice against infection. The drugs were active not only after oral but also after subcutaneous and intraperitoneal application. Rather an antagonistic effect was observed when the compounds were used combined. However treatment of mice with Tilorone HCl or a derivative in combination with Poly I:C enhanced survival of mice.

Zusammenfassung

Vier Derivate des Tilorone HCl induzierten Interferon und schützten Mäuse vor der Infektion mit dem FSME-Virus. Diese Substanzen waren nicht nur oral sondern auch s.c. und i.p. angewandt wirksam. Die kombinierte Gabe der Präparate zeigte eine antagonistische Wirkung. Die Kombination mit Poly I:C hingegen verstärkte den antiviralen Effekt der Tilorone HCl-Derivate deutlich.

TBE, spread by ticks, is a serious medical problem for many countries of Middle Europe (Kunz and Hofmann, 1973.) For instance, in Austria alone some 300-500 persons a year become ill in this way (Hofmann, 1973.) There is no specific therapy in practice yet against this infectious disease; to be sure, through experiments with animals by using interferon inductors such as Poly I:C (Kunz and Hofmann, 1971; Hofmann and Kunz, 1972; Hofmann, 1972) and Tilorone HCL (Hofmann and Kunz, 1972) a good protective effect was able to be attained. Certainly there

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1) Prof. Dr. G. Hennenberg, dedicated to him on his 65th birthday

2) Carried out with the support of Austrian funds for the advancement of scientific research.

is great hesitation about using these preparations on human beings. Poly I:C is surely too toxic for this purpose (Phillips and coworkers, 1971; Leonard and coworkers, 1969; Ostler and coworkers, 1970; Gralnick and coworkers, 1972), and although Tilorone HCL has the advantage of being a lower molecular substance, which is also highly effective when given orally, it is likewise not suitable for human beings (Zbinden and Emch, 1972; Rohovsky and coworkers, 1970; Kaufman and coworkers, 1971.) The Tilorone molecule has therefore been modified so as to develope in this manner substances which are more compatible.

The following is a report on the anti-viral effects of four such derivatives, in comparison with Tilorone HCL, against TBE in white mice.

Material and Methods:

1. Mice: The rodents chosen for this experiment (GP Swiss stock) weighed 10 grams and were bred by the Hygiene Institute of the University of Vienna.

2. Virus: TBE virus, "Hypr" 4 stock, baby mice breeding farm, Vienna.

3. Interferon inductors: Tilorone HCL and its derivatives (see diagram 1 below) were furnished to us by the Merrel Chemical Company, for which we are much indebted to Dr. R. Krueger. The substances were dissolved in distilled water. Poly I:C was obtained from the Miles Chemical Company and dissolved in PBS.

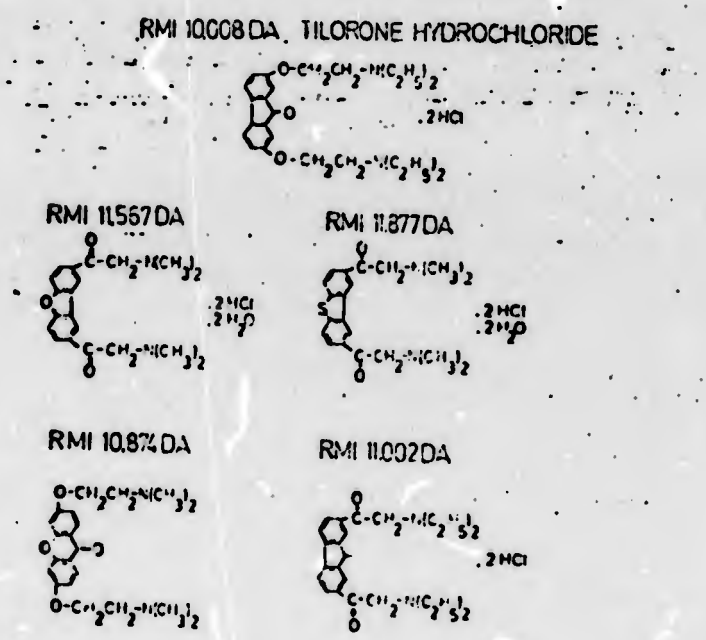


Diagram 1. Tilorone HCL derivative. (The diagram was very kindly placed at our disposal by the Merell National Laboratories.)

Antiviral Effects

4. Experimental planning: In the experiments, groups of 50 mice each were treated with the individual interferon inductors and, at the same time, a control group likewise of 50 rodents, not treated, were infected. The treatment dosage, infection dosage, the means of application and the method of treatment were varied in individual experiments (see under "results.")

5. Interferon demonstration: Interferon in serum from mice that were treated with one of the interferon inductors was evaluated in cultures in separate tubes against vesicular stomatitis (VSV.) This method has already been fully described elsewhere (Hofmann and co-workers, 1969.)

6. Statistical evaluation: The differences between individual groups in similar experiments were examined in the X^2 Significance Test, as described by Cavalli-Sforza (1969.)

Results:

1. Induction of interferon serum by Tilorone HCL and its Derivatives.

Each group of 20 mice were treated with Tilorone HCL and its four derivatives, orally, by means of a probang in doses of 250mg/kg and 16, 20, 24 and 40 hours afterwards blood was drawn off by punctures in the corner of the eye. As may be seen from Table No. 1, all 5 preparations induced interferon. RMI 11002DA proved to be the weakest.

Table No. 1: Induction of interferon serum:

Inductor	Interferon standard strengths of solution (reciprocal value) after:			
	16 hours	20 hours	24 hours	40 hours
Tilorone HCL	320	1280	640	80
RMI 11002 DA	80	160	320	20
RMI 11877 DA	320	640	320	40
RMI 11567 DA	640	1280	1280	40
RMI 10874 DA	320	640	1280	40

2. Effect of Tilorone HCL and its derivatives on TBE in white mice:

Groups of 50 mice weighing 10 grams each were treated orally by probang with Tilorone HCL and its derivatives in dosages of 250mg/kg per mouse, and 24 hours later given the same treatment, as well as a control group likewise of 50 rodents infected with 120 LD_{50} of TBE virus. As may be seen from Table No. 2, all of the control group mice died from the infection, whereas 16 of the mice treated with Tilorone HCL survived. The numbers of surviving mice from the groups treated with individual derivatives were as follows: RMI 11877: 4; RMI 11002: 8; RMI 10874: 10; RMI 11567: 10 (each RMI number is followed by "DA.)

3. Effect of the Combination of Tilorone HCL and its Derivatives.

In this experiment a group of 50 mice were treated with a mixture through two inductors (125 mg/kg per mouse) orally 24 hours before the infection with 27

LD_{50} of the TBE virus. As the extent of the experiment was limited by the number of mice available, all theoretically possible combinations could not be effected. For purposes of control, a group of 50 rodents was treated with a single inductor, in dosages of 250 mg/kg.

As may be seen from Table No. 3, the therapeutic effect through the combined application of the substances could not be improved. On the contrary, the combination showed a lesser effect than would have been expected from the addition of individual effects. Thus, 34 out of 50 mice treated with Tilorone HCL in dosages of 250 mg/kg survived; there were 32 out of 50, treated with RMI 11877 in dosages of the same amount, that survived. On the other hand, of each similar group that was given only half dosages of both substances, only 20 mice survived the infection. These differences between the result obtained from treatment with a single substance and the result of treatment with a combination of substances are statistically significant (See Table 3.)

4. Effect of Tilorone HCL and its Derivatives after subcutaneous and intraperitoneal application.

Previous experiments made it clear that there was a substantially higher toxicity in the case of subcutaneous and intraperitoneal application than after oral application. On this account, in this experiment the dosage was 100 mg/kg per mouse.

Table No. 4 shows that treatment by subcutaneous and intraperitoneal application is effective antivirally. Better effects were obtained by subcutaneous application.

5. Effect of very large doses of Tilorone HCL and its Derivatives.

As previous experiments had established that preparations fed by means of probangs were only mildly toxic for mice, their antiviral effect in case of higher dosages of 1,000 mg/kg was investigated.

The results of this experiment, which was carried out with 36 LD_{50}, are comprised in Table No. 5. It will be seen that in the cases of higher dosages, the differences between Tilorone HCL and its derivatives, as was observed for the lower dosages, are increased.

6. Effect of Tilorone HCL and its Derivatives in Combination with Poly I:C

In this experiment, which was conducted with 61 LD_{50} of the TBE virus, 26 hours after administering Tilorone HCL and one of its derivatives (i.e. two hours after the infection),a Poly I:C treatment(10 mg/kg) was added. Although this dose of Poly I:C did not, of itself, show any antiviral effect, it caused an improvement of the effect of Tilorone HCL, as may be seen from Table No. 6.

As there were not enough mice weighing 10 grams available at any one time, in this experiment we had to do without the control group, which would have been treated with Tilorone HCL derivatives only. But even without such control, the results were conclusive that Poly I:C strengthened not only the effect of Tilorone HCL but at least also that of RMI 10874 DA and RMI 11567 DA. Both substances were combined with Poly I:C in this experiment with Tilorone HCL, the effect of which they never before attained in previous experiments.

Table No. 2 Effect of Tilorone HCL and its Derivatives on TBE (120 LD_{50}) in White Mice

Combination	No. infected rodents surviving	Statistical Computation
Tilorone HCl	50/16	n.s.
RMI 11002 DA	50/8	1%
RMI 11877 DA	50/4	n.s.
RMI 11567 DA	50/10	n.s.
RMI 10874 DA	50/10	1%
Control	50/0	n.s.
		n.s.
		n.s.
		5%
		n.s.
		n.s.
		n.s.
		n.s.
		5%
		5%

n.s. = not significant

5% = significant (probability of error less than 5%)

1% = significant (probability of error less than 1%)

Table No. 3 Effect of th Combination of Tilorone HCL and its Derivatives

Treatment	No. infected rodents surviving	Statistical Computation
Tilorone HCl 250 mg/kg	50/34	
RMI 11567 DA 250 mg/kg	50/25	
RMI 11002 DA 250 mg/kg	50/20	
RMI 11877 DA 250 mg/kg	50/32	
RMI 11877 DA 125 mg/kg + RMI 11002 DA 125 mg/kg	50/19	
RMI 11877 DA 125 mg/kg + Tilorone HCl 125 mg/kg	50/20	
RMI 11002 DA 125 mg/kg + Tilorone HCl 125 mg/kg	50/19	
RMI 11567 DA 125 mg/kg + Tilorone HCl 125 mg/kg	50/21	
No control group	50/1	

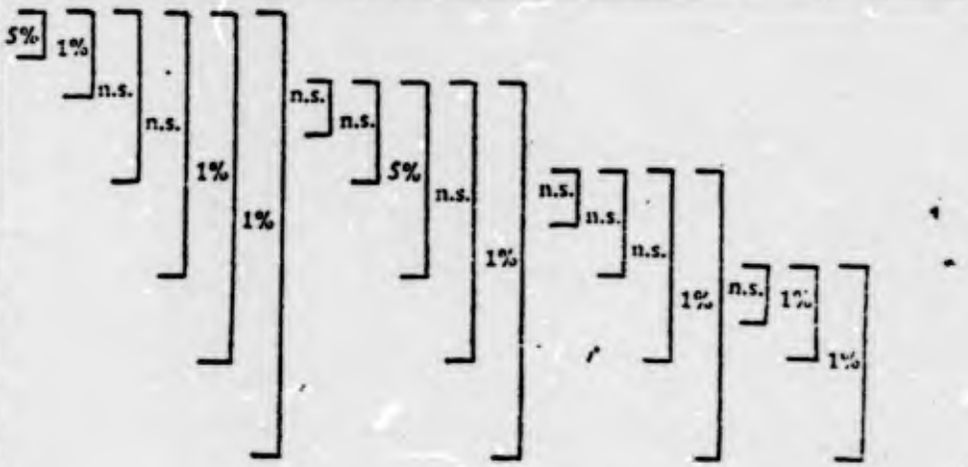
n.s. = not significant

5% = significant (probability of error less than 5%)

1% = significant (probability of error less than 1%)

Table No. 4 Effect of Tilorone and its Derivatives after subcutaneous and intraperitoneal application

Combination Application		No. infected rodents surviving	Statistical Computation
Tilorone HCl	s.c.	50/35	
	i.p.	50/24	
RMI 11002 DA	s.c.	50/20	
	i.p.	50/15	
RMI 10874 DA	s.c.	50/25	
	i.p.	50/21	
RMI 11877 DA	s.c.	50/33	
	i.p.	50/31	
RMI 11567 DA	s.c.	50/15	
	i.p.	50/15	
Control Grp		50/6	

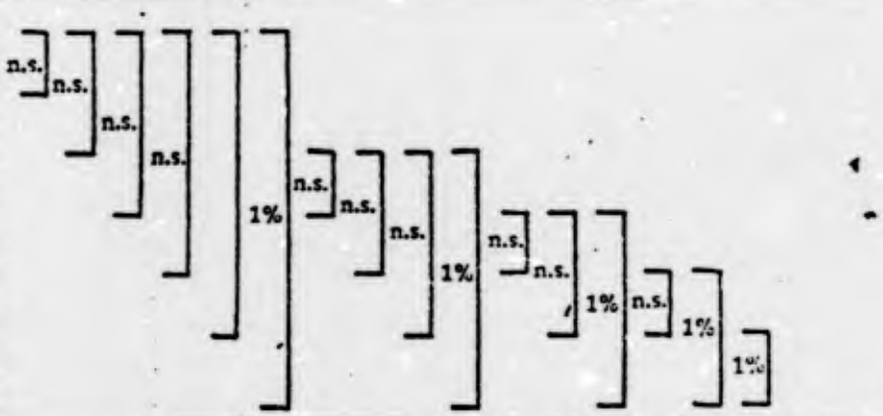


n.s. = not significant

5% = significant (probability of error less than 5%)

1% = significant (probability of error less than 1%)

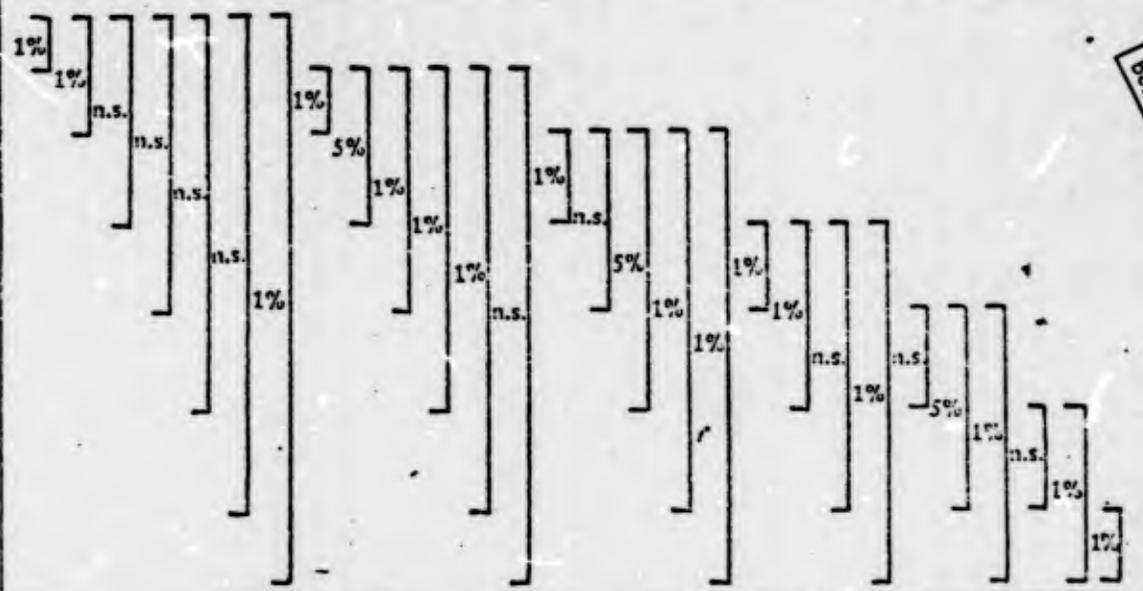
Table No. 5 Effect of Tilorone HCL and its Derivatives in very large dosages

Combination	Dosage	No. infected rodents surviving	Statistical Computation
Tilorone HCl	1000 mg/kg	47/29	
	500 mg/kg	50/23	
RMI 11002 DA	1000 mg/kg	47/23	
RMI 10874 DA	1000 mg/kg	46/26	
RMI 11567 DA	1000 mg/kg	48/21	
RMI 11877 DA	1000 mg/kg	50/31	
Control	1000 mg/kg	50/2	

n.s. = not significant

1% = significant (probability of error less than 1%)

Table No. 6 Effect of Tilorone HCL and its Derivatives in Combination with Poly I:C

Treatment	No. infected rodents surviving	Statistical Computation
Tilorone HCl 250 mg/kg	50/16	
Poly I:C 10 mg/kg	50/0	
Tilorone HCl 250 mg/kg + Poly I:C 10 mg/kg	50/31	
RMI 11002 DA 250 mg/kg + Poly I:C 10 mg/kg	50/7	
RMI 10874 DA 250 mg/kg + Poly I:C 10 mg/kg	50/23	
RMI 11567 DA 250 mg/kg + Poly I:C 10 mg/kg	50/20	
RMI 11877 DA 250 mg/kg + Poly I:C 10 mg/kg	50/12	
No control group	50/0	

n.s. = not significant

5% = significant (probability of error less than 5%)

1% = significant (probability of error less than 1%)

Antiviral Effects

Discussion

Our experiments show that Tilorone HCL has an antiviral effect against TBE in white mice when applied with the tested derivatives. Certainly no combination achieved a better effect than Tilorone HCL itself, in which connection no significant differences were found among the four derivatives. Also, the standard strengths of free interferon serum inducible through the preparations were ranked pretty much the same; only RMI 11002 DA induced noticeably less interferon than the other combinations (Table 1.) As this substance had also only slight antiviral effects in other experiments (Tables 2 and 3), whenever there was a correlation between interferon induction and antiviral effect, processes other than interferon induction should not be decisively effective.

It is striking that the combination of the preparations resulted in even a significantly lesser antiviral effect. This could be traced back to a decidedly quick reabsorption of the individual derivatives. Possibly the more quickly reabsorbed substance produced a so-called hypo-reactive condition in the cells for the succeeding induction of the second slowest substance tried. Differences in reabsorption could also explain the fact that the increase in oral dosages to 1000 mg/kg per mouse, in the main, caused any differences between Tilorone HCL on the one hand and its derivatives on the other, to practically vanish.

In contrast to the combination of the preparations with one another, the treatment together with Poly I:C causes an improved effect. As in this experiment (Table 6) the Poly I:C dosage alone remained uneffective, it can hardly be a case of additive effect. Possibly the pre-treatment of the rodents with Tilorone HCL and its derivatives created a "priming" effect for the interferon stimulation to follow, through Poly I:C, through which increased interferon was built up. The fact that in one case (experiment table 3) a "hypo-reactive condition" for the succeeding interferon induction was caused, and in another case (experiment table 6) there was created a "priming" and thereby a better interferon buildup after the second inductor was used, is to be explained, on the one hand by the variable interferon inductors, but on the other hand, by the time difference between the applications of the inductors. The meaning of the time interval, in this connection, is also evidenced by an experiment formerly conducted by us (Hofmann and Kunz, 1971.) On that occasion we also combined Tilorone HCL and Poly I:C and we found only an insignificant improvement in the interferon reaction to Poly I:C after the previous Tilorone HCL treatment. On the contrary, the strengthening of the antiviral effect that was manifest in the number of surviving rodents was not evident. In the experiments conducted at that time, the time interval between the administering of Tilorone HCL and Poly I:C was only a few hours longer than in the present experiment.

In any case, however, we now see how to develope Tilorone HCL derivatives, hopefully to arrive at combinations which can also be applied to human beings. TBE would be an important indication area for such preparatives.

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