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PHARMACODYNAMIC EVALUATION OF THE PERIPHERAL PAIN INHIBITION BY CARPROFEN AND FLUNIXIN IN THE HORSE

U. SCHATZMANN¹, M. GUGELMANN¹, J. VON CRANACH¹, B. M. LUDWIG², W. F. REHM²

SUMMARY

Carprofen, flunixin meglumine and placebo in the form of a physiological solution of sodium chloride were tested in an open randomised cross-over trial for analgesic efficacy in horses with two external skin-stimulation systems. Both systems, the withers model and the "heating element" model, were compared in order to find an optimal way to measure pain perception after stimulating the skin with high temperature.

No analgesic effect of flunixin or carprofen could be demonstrated when using the withers model. In the "heating element" model, a 1.1 mg/kg i. v. dose of flunixin meglumine failed to inhibit the peripheral pain, while it could be shown that a 0.7 mg/kg i. v. dose of carprofen inhibited the peripheral perception of pain in horses for approximately 24 hours after the drug injection. To induce an analgesic effect with carprofen, its plasma concentration had to be at least 1.5 µg/ml.

PHARMAKODYNAMISCHE UNTERSUCHUNG DER HEMMUNG DES PERIPHEREN SCHMERZES DURCH CARPROFEN UND FLUNIXIN BEIM PFERD

Die analgetische Wirkung von Carprofen, Flunixin-Meglumin und einem aus physiologischer Kochsalzlösung bestehenden Plazebo wurde in einem offenen, randomisierten Überkreuz-Versuch bei Pferden in zwei Versuchsmödellen geprüft. In beiden Modellen wird der Schmerzreiz durch externe Stimulation der Haut gesetzt.

Beide Versuchsmödelle, das Widerrist-Modell und das «Heizelement»-Modell, wurden miteinander verglichen, um den besten Weg zur Messung einer Schmerzreaktion nach Stimulation der Haut durch erhöhte Temperatur zu finden.

Im Widerrist-Modell zeigten weder Flunixin noch Carprofen eine analgetische Wirkung. Im «Heizelement»-Modell liess sich mit Flunixin-Meglumin (Dosis: 1,1 mg/kg Körperegewicht (KGW) intravenös) eine Hemmung des peripheren Schmerzes nicht nachweisen. Mit Carprofen war es möglich, nach einer intravenösen Dosis von 0,7 mg/kg KGW eine Hemmung der Schmerzen bei den Pferden während einer Periode von etwa 24 Stunden nach der Injektion nachzuweisen. Um den analgetischen Effekt von Carprofen zu erzielen, müssen die Plasmakonzentrationen mindestens 1,5 µg/ml betragen.

KEY WORDS: horse — analgesy — Carprofen — Flunixin — plasma concentration

SCHLÜSSELWÖRTER: Pferd — Analgesie — Carprofen — Flunixin — Plasmakonzentration

INTRODUCTION

Carprofen is a non steroidal anti-inflammatory drug (NSAID) of the carboxylic acid type which possesses anti-inflammatory, analgesic and antipyretic actions (Randall and Baruth, 1976; Strub et al., 1982). The plasma elimination half-life of

carprofen in horses ($t_{1/2\beta}$) is approximately 20 hours (von Fellenberg et al., 1986; McKellar et al., 1989).

The aim of this study was to determine, with two different animal models, the pain threshold to external skin stimulation with heat in horses after treatment with carprofen in comparison to the commonly used NSAID flunixin meglumi-

ne and a placebo. Once the pain threshold is known, it is possible to assess the analgesic efficacy of the tested drugs. The effects of carprofen, flunixin-meglumine and placebo on the cardio-respiratory system and on various haematological parameters were also evaluated, and will be reported by Gugelmann (1989) in a separate paper.

ANIMALS, MATERIAL AND METHODS

Test system

For the study, 7 clinically healthy horses were used. No medicinal treatment was given for two weeks before the experiments. The eldest horse, which was 21 years old and of Irish origin, with a body weight of 585 kg, was used in a pilot trial to validate the models of the experiment. The other six horses (Nos. 1–6) were 3 year old Swiss riding horses, with body weights between 520 and 620 kg.

Test substances

The test substances were in the form of injectable preparations.

- *Carprofen* (INN) was given as a 5% mixed micelle solution in ampoules containing 3 ml with 50 mg active substance per ml (code No. Ro 20-5720/656, lot No. GPH 13 027).
- *Flunixin meglumine* was given in the form of the commercially available injectable preparation (trade name: FINADY-NE®, Biokema, CH-1023 Crissier/Switzerland (lot No. 8511)).
- The *placebo* was given in the form of a sterilized physiological solution of 0.9% sodium chloride in vials with 100 ml each.

The injection of each of the three products was carried out within 20 sec into the right jugular vein of the animals. The doses were as follows:

Carprofen	0.7 mg/kg b. w.
Flunixin meglumine	1.1 mg/kg b. w.
Placebo	0.2 mg/kg b. w.

The flunixin dose was as recommended by the manufacturer, the dose of the placebo was chosen to reach a similar injection volume to the carprofen dose.

Trial design

The experiments were performed as an open cross-over trial. The horses were treated with all three products according to table 1. The tests were performed with a wash-out period of 1 week between each treatment.

Table 1: Trial design

Horse No.	Treatment		
	1st experiment	2nd experiment	3rd experiment
1	C	F	P
2	F	P	C
3	P	C	F
4	C	P	F
5	P	F	C
6	F	C	P

C = carprofen

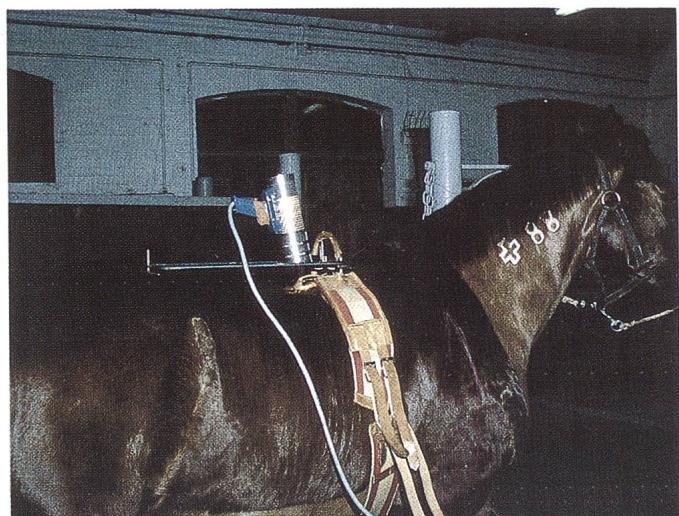
F = flunixin meglumine

P = placebo

Evaluation of the pain threshold (cutaneous pain perception)

Withers model: As in the method described by Kamerling et al. (1985) and Vögeli (1988) a halogen lamp was attached to a fixed bar above the withers of the horse. The light was focussed on a clipped area of the withers (fig. 1). The light was turned on by remote control and the reaction of the horse observed. The time (in seconds) until the first pain reaction, visible by definite signs of discomfort, was measured using a stopwatch. The light was then immediately switched off.

Fig. 1: Withers model

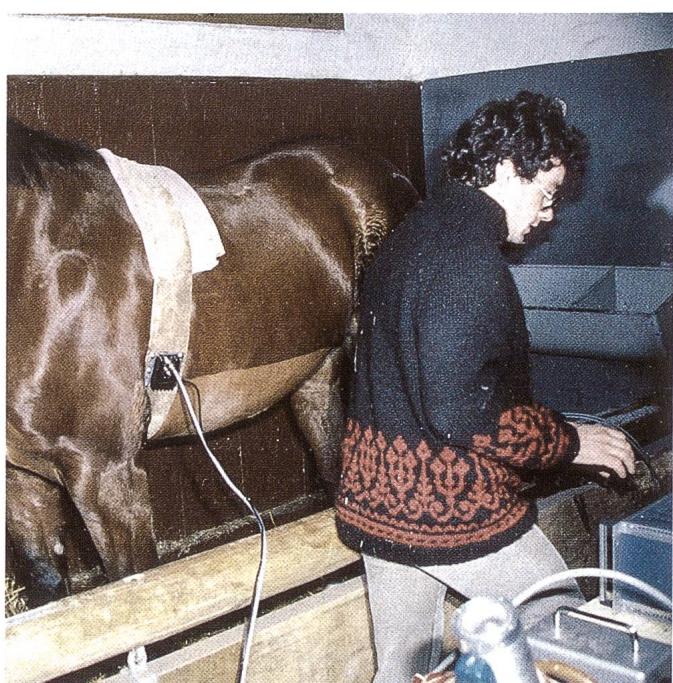


The skin twitch reflex or a clear defense reaction was easily identifiable as a measure of cutaneous pain threshold. When no reaction occurred after 30 sec., the stimulation was stopped. Three consecutive measurements were performed at every point of time. The arithmetic mean value was calculated.

Pain perception using this technique was tested before treatment and at the following observation times after injection of the test substances: 30 and 60 min, 6, 12 and 24 hr.

Stimulation with the heating element: The specially designed apparatus for this model consisted of a heating element (diameter 1.5 cm) fixed on a girth and applied tightly on a clipped area behind the elbow (fig. 2) with the horse restrained in stocks. The element is connected to a display and power unit with a cable (2 meters). The apparatus allowed the element temperature to be increased gradually (1°C every 5 sec) and the actual temperature to be displayed without the horse being touched.

Fig. 2: «Heating element» model



The threshold temperature, as expressed by lifting a leg, looking backwards or attempts to approach the element with the mouth was recorded. After stopping, the heating element was actively cooled. Three consecutive measurements were performed at each measuring site and the arithmetic mean of the tolerated temperature was calculated.

Pain perception using this technique was tested before treatment and 30, 60 min, 6, 12 and 24 hr after injection of the test substances.

Criteria for the assessment of analgesic activity

In order to assess whether or not the tested drug exhibited an analgesic effect, the criteria for activity has to be defined for each of the two models.

Withers model: In this model, the time until a reaction occurred to a constant heat stimulus was measured.

Before any treatment (either placebo, flunixin meglumine or carprofen) had been given to the horses, the basic time until a reaction to the heat occurred, was measured (control values). As baseline, the mean reaction-time was calculated from 17 measurements carried out before treatment during each of the three experiments described in table 1.

In our investigations, the control reaction was equal to 7.1 seconds. The standard error of the mean was equal to 0.8 seconds corresponding to a 95% confidence interval of 1.7 seconds. Horse No. 4 showed an unexpectedly long reaction time (17 to 30 seconds) in the first experiment and was therefore not included in this calculation.

An analgesic action due to the treatment in question was defined as follows:

- the reaction time measured in a horse treated with either placebo, flunixin meglumine or carprofen, had to be, *at least, 2 seconds longer* than the value measured in the horse in question before the given treatment;
- the increase of at least 2 seconds in the reaction time had to be observed *at two successive observation times* after treatment.

“Heating element” model: In this model, the temperature tolerated by the horses during a standard time was determined. Before any treatment had been given to the horses, the baseline temperature until a reaction to the heat occurred, was measured (control values). In our investigations, the basic temperature (18 measurements) was equal to 45.60 °C. The standard error of the mean was 0.46 °C which corresponded to a 95% confidence interval of 0.98 °C.

The analgesic effect due to a given treatment was defined as follows:

- the tolerated temperature measured in a horse after treatment with either placebo, flunixin meglumine or carprofen, *had to be increased by 1 °C* in comparison to the control temperature in the horse in question;

- this increase of the tolerated temperature had to be observed, *during at least, three successive observation times* after the treatment.

Determination of carprofen concentration in blood plasma

From all horses treated with carprofen, 5 ml blood was taken before injection and at the subsequent observation times after carprofen injection (30 min, 1, 6, 12 and 24 hr). The blood was drawn into ammonium-heparin containing syringes (Monovettes, Sarstedt). After mixing, the blood was transferred into glass tubes and immediately centrifuged (5 min, 1500 g). The plasma was stored at -20°C until the analytical investigations were carried out. The determination of carprofen in the plasma was carried out by means of a high performance liquid chromatographic method as described by *Ascalone* and *Dal Bó* (1983). The quantification limit of the method was equal to 0.025 $\mu\text{g}/\text{ml}$, and its accuracy and precision was better than 15% within a concentration range of 0.025–10.0 $\mu\text{g}/\text{ml}$.

RESULTS

Withers model

The mean times until the horses reacted to the heat stimulus produced by the lamp are listed in table 2. One horse was not included in the calculation of the pain threshold because the values measured before and after treatment were unexpectedly high (≥ 15 seconds). The mean ($n=5$) difference between the reaction times measured before and after each treatment were calculated. The curves of the values calculated at various times after the injection of carprofen, flunixin meglumine and placebo are shown in figures 3 and 4.

As can be seen from these figures, the reaction times measured after either placebo, flunixin or carprofen were below the defined level for analgesic activity, i. e. no analgesic effect of flunixin or carprofen could be clearly demonstrated with the withers model.

Stimulation with the heating element (`heating element' model)

The mean ($n=6$) tolerated temperatures measured after each treatment are given in table 3.

The differences between the tolerated temperature before and after the treatment was given to the horses, were calculated for each animal. The curves of the differences of tolerated temperatures calculated at various times after either carprofen, flunixin meglumine or placebo are shown in figures 5 and 6.

Fig. 3: Mean (+/– SEM, $n=6$) of the difference of reaction time (seconds) after flunixin meglumine or placebo treatment in horses

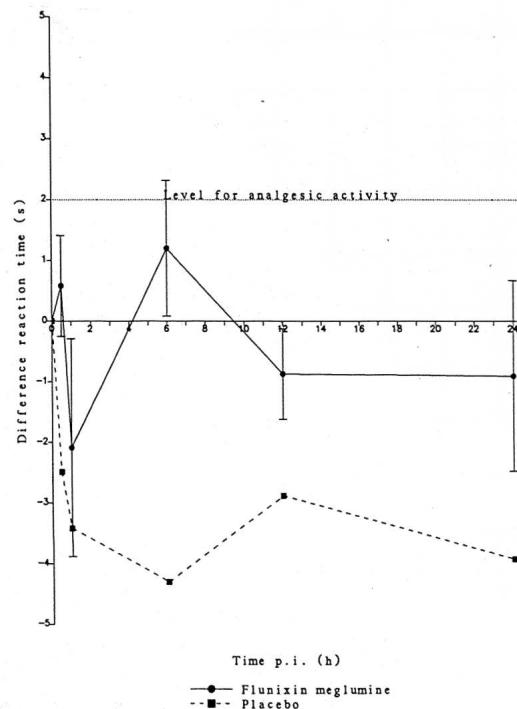


Fig. 4: Mean (+/– SEM) of the difference of reaction time (seconds) after carprofen ($n=5$) or placebo ($n=6$) treatment in horses

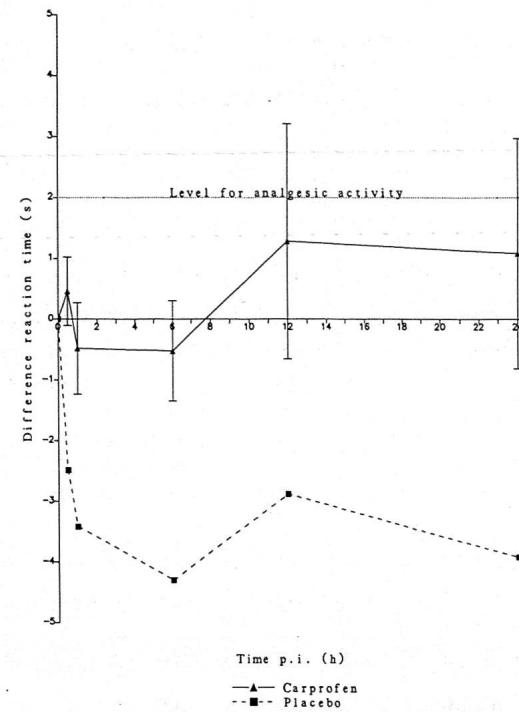


Table 2: Mean reaction time in seconds (\pm SEM) after treatment with carprofen, flunixin meglumine and placebo

Treatment	Number of horses	Time of testing before and after treatment (hr)					
		before	0.5	1	6	12	24
Carprofen	5*	5.3 (1.0)	5.8 (0.8)	4.9 (0.7)	4.8 (0.7)	6.6 (1.3)	6.4 (1.1)
Flunixin meglumine	6	7.3 (1.3)	7.8 (1.8)	5.2 (1.8)	8.5 (2.2)	6.4 (1.4)	6.4 (1.8)
Placebo	6	8.4 (1.5)	5.9 (1.1)	4.9 (0.8)	4.7 (0.4)	4.6 (0.7)	4.4 (0.7)

* One horse was considered to be an outlier in this study

Table 3: Mean tolerated temperature in °C (\pm SEM) after treatment with carprofen, flunixin meglumine and placebo

Treatment	Number of horses	Time of testing before and after treatment (hr)					
		before	0.5	1	6	12	24
Carprofen	6	44.5 (0.5)	45.6 (1.2)	46.4 (1.1)	47.0 (1.1)	46.9 (1.4)	46.1 (1.2)
Flunixin meglumine	6	45.9 (0.8)	47.4 (2.3)	46.5 (1.8)	45.9 (1.5)	45.3 (1.0)	43.2 (1.1)
Placebo	6	46.3 (0.9)	46.7 (1.2)	46.9 (1.6)	45.7 (0.5)	46.5 (0.6)	44.0 (1.2)

The mean difference of the tolerated temperature calculated after the injection of flunixin meglumine was higher than 1 °C at 0.5 hour after the drug injection. At the observation times 1 hour and later, the values were below 1 °C, and in the range of the temperature difference calculated following placebo treatment (fig. 5).

The mean differences of the tolerated temperature calculated after carprofen treatment were higher than 1 °C for the whole observation period of 24 hours p. i. This indicated that carprofen, according to the defined criteria, exhibited analgesic activity at the 0.7 mg/kg b. w. dose level over a period of 24 hours after treatment.

When looking at the results in each individual horse, it could be seen that flunixin demonstrated an analgesic effect in one of the six horses treated, whilst carprofen had an analgesic effect in four of the six horses treated.

Pharmacokinetic values

A 2-exponential equation, corresponding to an open 2-compartment model, could be fitted to the plasma concentration-

time data of carprofen by extended least squares non-linear regression analysis by using the ELSFIT programme (Steiner and Beal, 1985).

With this programme, the plasma elimination half-life was found equal to approximately 20 hours and the volume of distribution V_B was less than 0.25 l/kg. These results are in agreement with what was found previously (von Fellenberg et al., 1986; McKellar et al., 1989).

DISCUSSION

Withers model versus «heating element» model

The criterion for the withers model is the time taken until pain is produced by the standard stimulus (Kamerling et al., 1985). An analgesic effect of flunixin or carprofen could not be clearly demonstrated with the withers model because the reaction time for all three products were below the defined level for an analgesic activity. Moreover, the difference of the reaction-times before and after placebo treatment were always negative from 0.5 hour up to 24 hours after the placebo

Fig. 5: Mean difference of tolerated temperature (+/- SEM, n = 6) by the horses before and after either flunixin or placebo treatment («heating element» model)

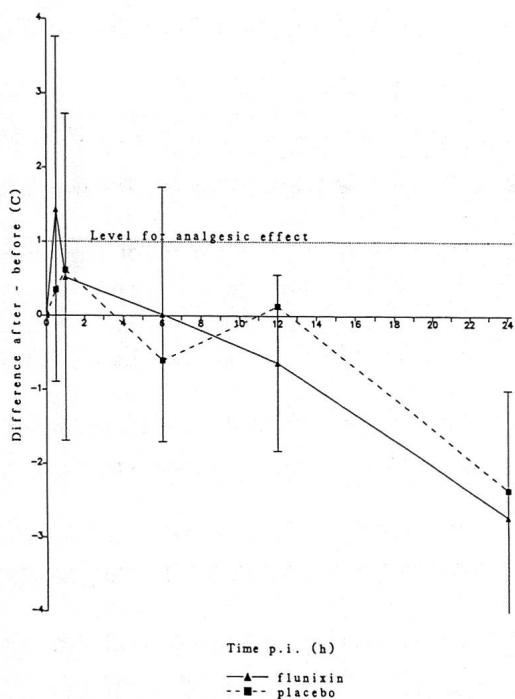
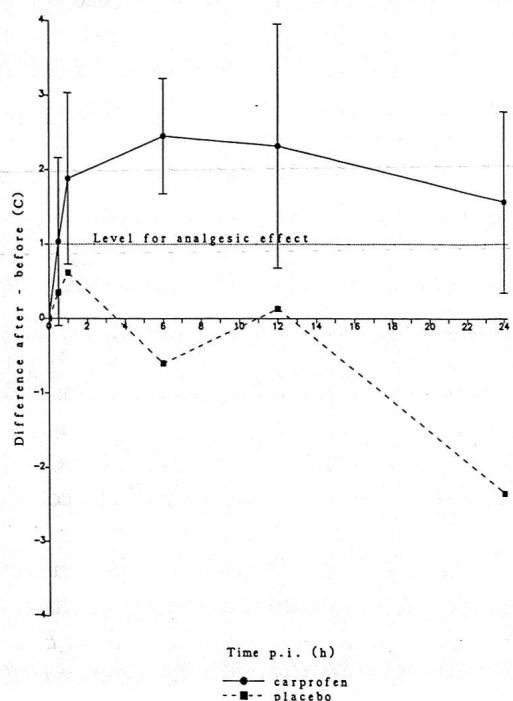


Fig. 6: Mean difference of tolerated temperature (+/- SEM, n = 6) by the horses before and after either carprofen or placebo treatment («heating element» model)



injection. This shows that the reaction times measured after the first three stimulations carried out before treatment, became stepwise shorter, suggesting that a learning effect may occur during the experiments. This phenomenon may be due to the fact that the horses perceived the flash of the burning lamp. This learning effect (fig. 3 and 4) probably masked the reaction to the pain and made it impossible to evaluate the pain threshold. Therefore, the time until a pain reaction can be measured (reaction time) does not seem to be an adequate parameter to assess the analgesic effect.

Nevertheless, the fact that the profile of the curves obtained after either flunixin or carprofen treatment differed from that obtained after placebo treatment (fig. 3 and 4), may lead to the conclusion that both carprofen and flunixin had certain analgesic properties. But with the withers model, objective results could not be reached.

The «heating element» model showed more precise results than the withers model. A «learning effect» could not be observed (fig. 5 and 6). This may be confirmed by the fact that the values calculated after placebo treatment are scattered around 0 °C. The reason may be that in the case of the «heating element» model the increase of the temperature is obtained with the help of a heating device which prevents the horse from learning whether, and when, pain will occur. Therefore, the assessment of even a slight analgesic effect was possible. In addition to this, a comparison of the analgesic efficacy of the three products was made.

Carprofen versus flunixin meglumine

With the «heating element» model, we could demonstrate that with the recommended dose of 1.1 mg/kg b. w., flunixin meglumine failed to inhibit peripheral pain. Only in one horse, did flunixin meglumine show a short lasting (≤ 1 hour) analgesic activity. Flunixin is known as a good analgesic, but on the other hand this NSAID is rapidly excreted from the body of horses, its plasma elimination half-life being less than 2 hours (Soma et al., 1988). Furthermore, it may be assumed that in the case of the inhibition of peripheral pain, there is probably no possibility of a drug accumulating at the site of the pain as was shown to occur with phenylbutazone or flunixin in the exudate of sites of inflammation (Lees et al., 1987). Since flunixin is rapidly eliminated from the body of horses, an analgesic effect can only last for a short time, or even not take place at all. Therefore, the increased temperature tolerance at 0.5 hour after treatment may possibly be explained by a short lasting analgesic effect of flunixin.

Regarding carprofen, it could be shown that at the 0.7 mg/kg b. w. dose level, carprofen did inhibit the peripheral percep-

tion of pain as demonstrated by the higher tolerated temperature measured after the injection of this NSAID, compared to the temperature measured before the carprofen injection. This inhibition was observed, on an average, for 24 hours after the drug injection.

Relationship of plasma concentration of carprofen and analgesic effect

The analgesic acitivity of carprofen was shown to last up to 24 hour after the drug injection. The time during which analgesia could be measured, corresponds to the long plasma elimination half-life of 20 h in horses.

Fig. 7: Plot of the mean difference of tolerated temperature (\pm SEM, $n = 6$) by the horses, against concentration of carprofen in plasma («heating element» model)

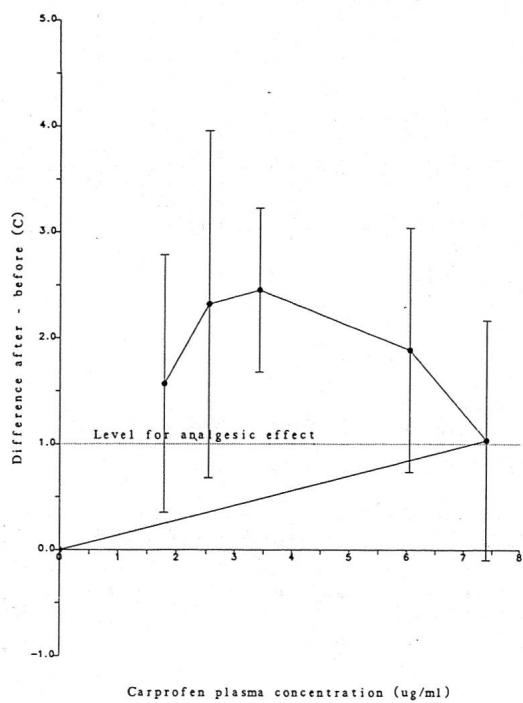


Figure 7 shows the plot of the analgesic effect of carprofen against the mean plasma concentration of intravenously administered carprofen (0.7 mg/kg b. w.). The anticlockwise hysteresis loop which is obtained, is characteristic of an equilibration delay. It also indicates that there is no direct relationship between the total (free and protein-bound) concentration of the drug in plasma and its effect (Holford and Steiner, 1981). Rather, the analgesic effect of carprofen is related to the concentration of the drug at the site of action, this concentration being in turn dependent on the plasma concentration of carprofen. It can be seen from figure 7, that

the total plasma concentration of carprofen should be, on an average, equal to, or higher than, 1.5 μ g/ml in order to observe an analgesic effect of the drug in horses. A higher dose could possibly extend the duration of the analgesia.

CONCLUSION

The «heating element» model proved to be more precise for the assessment of the analgesic effect of NSAIDs than the withers model. If the blood plasma concentration of carprofen is compared with the analgesic effect, the minimum concentration of carprofen in plasma, which produces inhibition of pain, could be determined, and was found equal to approximately 1.5 μ g/ml. This concentration could be reached for approximately a 24-hour period of time after the intravenous injection of a 0.7 mg/kg b. w. dose. If the time of pain inhibition has to be extended over 24 hours, then a higher dose of carprofen, or a daily drug administration is needed.

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Evaluation pharmacodynamique de l'activité analgésique du carprofène et de la flunixine chez le cheval

Le Carprofène et la Flunixine méglumine ont été testés contre un placebo (solution injectable de sérum physiologique) pour leur activité analgésique chez le cheval, lors d'un essai croisé ouvert et randomisé.

Afin d'évaluer de manière optimale la perception de la douleur provoquée par une élévation de la température au niveau de la peau, deux modèles ont été retenus: le modèle usuel «withers model» d'une part, et un nouveau modèle que nous avons développé, le «heating element model» d'autre part. Aucune activité analgésique n'a pu être démontrée pour la flunixine comme pour le carprofène dans le modèle usuel («withers model»). Dans notre modèle («heating element model») une dose intraveineuse de 1,1 mg/kg de flunixine méglumine n'a pas réduit la perception de la douleur périphérique. Par contre, dans ce même modèle, le carprofène permet de diminuer la sensation de la douleur périphérique à une dose de 0,7 mg/kg par voie intraveineuse, et ce durant environ 24 h chez le cheval. Cet effet analgésique du Carprofène est observable pour des concentrations plasmatiques minimales de 1,5 μ g/ml.

Valutazione farmacodinamica del dolore periferico. Inibizione con il caprofen e con la flunixina nel cavallo

L'effetto analgesico del caprofen e della flunixina meglumina e di un placebo contenente soluzione fisiologica è stato esaminato sui cavalli in due esperimenti modello incrociati, aperti e randomizzati. In tutti e due i modelli il dolore viene inflitto mediante stimolazione esterna della pelle.

Tutti e due i modelli, il «Withers-model» e il «heating element model» furono paragonati fra di loro per trovare la via migliore per la misurazione del dolore dopo la stimolazione della pelle con alte temperature.

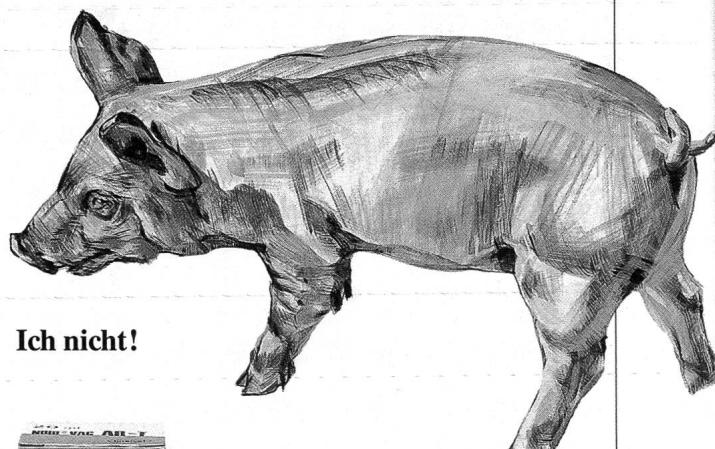
Nel «Withers-model» né la flunixina né il caprofen mostrano una azione analgesica. Nel «heating element model» con la flunixina meglumina (dose: 1,1 mg/kg i. v.) non si misura alcuna inibizione del dolore periferico. Con il caprofen invece, fu possibile con un'iniezione i. v. (dose: 0,7 mg/kg) inibire

il dolore nei cavalli per un periodo di 24 ore dopo l'iniezione. Per ottenere un effetto analgesico col caprofen è necessario raggiungere una concentrazione plasmatica di 1,5 μ g/ml.

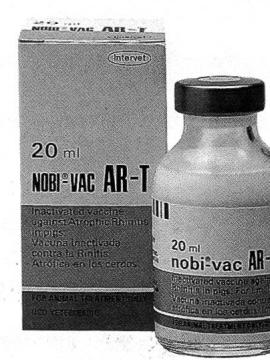
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