thermal expansion increased, and the diametric coefficient of expansion was not significantly different from Layer A ($P < 0.01$).

Rowe (1980) hypothesized that differences in the dimensional changes of the film and tablet substrate during film coating could play a role in the creation of film defects.

The formulations corresponding to the two layers were compressed individually and samples film coated. Cracking was not evident in the film-coated samples nor in the single-layer compressed tablets when subjected to temperatures of 40–55°C for 24 h.

Fractures appeared in uncoated bilayered tablets placed in a 40°C oven overnight, however, indicating that their exposure to elevated temperatures, rather than the application of a film coating itself, was the cause of tablet cracking. Separation of the layers was not observed. It is speculated that differences in dimensional changes between the two layers, when subjected to the heat encountered during film coating, created stress that was relieved by fracturing of the less thermally elastic layer (Layer B). Although the origin of the fractures could not be determined, they characteristically extended from the tablet surface vertically to the interface between the two layers (Fig. 2).

The successful manufacture of a bilayered tablet requires the development of a combination of formulations that can form interfacial bonds during the compressing operation sufficient to withstand subsequent stresses encountered during film coating, storage at elevated temperatures and physical handling. TMA provides a rapid, convenient and reproducible method of screening combinations which might prove suitable for use in this particular dosage form.

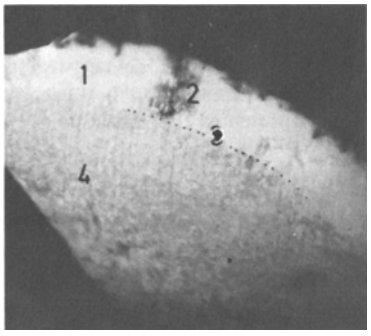

FIG. 2. Cracking in a film-coated bilayered tablet. Cross-section showing: 1 layer B, 2 vertical crack, 3 interface, 4 layer A.

References

Rowe, R. C. (1980) The expansion and contraction of tablets during film coating—a possible contributory factor in the creation of stress within the film. J. Pharm. Pharmacol. 32: 851

J. Pharm. Pharmacol. 1988, 40: 283–285
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Pharmacokinetic evaluation of local drug delivery: the intratesticular and intrarenal administration of acenocoumarol in the rat

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Abstract—According to theory, the regional increase in drug concentration during target organ directed drug delivery as compared with systemic drug delivery is related to the quotient of the clearance of the drug and the blood flow of the target organ. We investigated the steady-state pharmacokinetic disposition of *S*-acenocoumarol in plasma, liver, testis, and kidney following its administration (constant rate infusion by an osmotic minipump) directly into the testis or the kidney of rats. The effects of clearance induction (phenobarbitone treatment) on the disposition of the drug were also investigated. The results confirm the theory of target-directed drug delivery.

Recent findings in biochemical research on the molecular function of vitamin K made it clear that vitamin K-dependent biochemical systems not only are present in liver tissue but are also found in tissues like bone, kidney, testis, brain, endothelial cells, etc. (Hauschka et al 1976; Vermeer et al 1982). The physiological function of vitamin K in non-hepatic tissues is still unclear. An approach for obtaining more insight in this field would be to study the effects of the suppression of the local vitamin K systems. Oral anticoagulants, i.e. 4-hydroxycoumarins, interfere with the cellular vitamin K function by suppressing the enzyme vitamin K-epoxide reductase which is part of the cellular vitamin K cycle (Suttie 1980). These drugs, however, cannot be applied as such because, as we have shown recently (Thijssen et al 1986), they preferentially accumulate in liver tissue, thus giving only a weak response in non-hepatic tissues. Target-directed delivery of oral anticoagulants would be a method to circumvent this problem. According to theory, the advantage of direct drug delivery to a non-eliminating target organ over delivery via the systemic circulation is only obtained if the blood (plasma) flow of the target organ is low in comparison to the blood (plasma) clearance of the applied drug (Eckmann et al 1974; Chen & Gross 1980). We applied the technique of local drug delivery to investigate the function of the testicular vitamin K system in rats (Daemen et al unpublished). The *S*-enantiomer of acenocoumarol (AC) was used as the vitamin K 'antagonist' because its blood clearance (about 4 mL min^{-1}; Daemen et al 1986) is high with respect to testicular blood flow (0·2–0·4 mL min^{-1}; Nishiyama et al 1976).

Since to date experimental evaluation of the theory of local drug administration has not been presented in literature, we wish to report here the pharmacokinetic analysis of our experiments.

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We additionally tested the theory of target drug delivery by investigating the effect of induced clearance (phenobarbitone induction) and by investigating AC delivery to a high flow organ (i.e. kidney). The biochemical and biological effects of the experiments will be presented elsewhere.

Materials and methods

Wistar rats were purchased from Winkelman (Borchen, West Germany). Before and throughout the whole experiment the rats were on standard laboratory food and tap water.

Intratesticular administration. Male rats, aged 14–16 weeks, were used. Under light ether anaesthesia, a femoral artery and a testicular catheter were placed, for blood sampling and drug administration, respectively. The testicular catheter was placed as follows: the abdomen was opened by a midline incision of about 2 cm and the left testis retracted into the abdomen. A silastic tube (internal diameter 0·6 mm), was introduced into the left testis through a small incision of the capsule. The tip of the tube protruded into the centre of the testicular tissue. The tube was sutured and fixed on the testicular surface with tissue glue. The tube was filled with a sterile solution of AC (1 mg mL^{-1}) in 0·06 M phosphate buffer pH 8·0. The other end of the silastic tube was guided subcutaneously to the neck and attached to an Alzet osmotic minipump (model 2001, ALZA, Palo Alto, Ca.). The pump was inserted subcutaneously between the shoulder blades. The infusion rate of the pump was 1 $\mu L\ h^{-1}$. The femoral artery catheter (Polyethylene-10) was also led to the neck and closed with a metal plug. The experiments lasted for five days. Pilot experiments showed that AC administered at a rate of 1 $\mu g\ h^{-1}$ subcutaneously for several days caused no bleeding complications. Arterial blood samples (0·5 mL) were taken on the third, fourth and fifth day of infusion. On the fifth day blood was collected via the abdominal aorta from the animals under light ether anaesthesia then the animals were killed. Kidneys and liver were rinsed in-situ with ice-cold 0·9% NaCl (saline) and excised. Testis were removed and kept in ice-cold saline until homogenization (see later).

To study the effect of induced clearance, rats received phenobarbitone (1 mg mL^{-1}) in their drinking water starting 5 days before the experiment. Administration was continued via the drinking water during the experiment.

Intrarenal administration. AC was infused directly into the renal artery of the right kidney of male Wistar rats, aged 14–16 weeks. For that purpose, an elongated PE-10 catheter was introduced in a retrograde fashion in the right suprarenal artery following the procedure of Smits et al (1983). The other end of the catheter was led to the neck and attached to an osmotic minipump filled with AC (1 $\mu g\ \mu L^{-1}$). At the fifth day of infusion the animals were killed and kidneys, testes, liver and blood removed.

Analytical procedures Blood samples were mixed immediately with 0·1 M trisodium citrate (9/1, v/v). The blood samples were centrifuged and the plasma stored at −25°C until assayed. Liver and kidneys were homogenized in three volumes (w/v) of ice-cold buffer, 0·02 M Tris HCl, 0·15 M KCl, pH 7·4, using the Potter technique. Testis homogenates were prepared as follows: the testicular capsule was removed and the soft tissue was thoroughly rinsed in ice-cold saline. The tissue was carefully blotted dry and homogenized.

Tissue AC concentrations were analysed as described previously (Thijssen et al 1985).

Pharmacokinetic analysis. The systemic plasma clearance (mL min^{-1}) of AC was calculated by

$$Cl_S = inf/C_S$$

where inf equals the infusion rate (ng min^{-1}) and C_S the systemic arterial plasma concentration at steady state (ng mL^{-1}).

Results

Intratesticular infusion. The plasma concentrations of AC on the third, fourth and fifth day of infusion were 5·7 ± 0·7, 5·3 ± 0·5 and 5·3 ± 0·4 ng mL^{-1} (mean ± s.e.m., n = 9). Rats that were given phenobarbitone via the drinking water showed significant ($P < 0·001$) lower plasma acenocoumarol concentrations: 4 ± 1·7, 4·1 ± 1·5 and 3·7 ± 1·6 ng mL^{-1} (n = 7) on the third, fourth and fifth day, respectively. The results indicate a plasma clearance of AC of 3·4 ± 0·4 and 4·6 ± 0·3 mL min^{-1} ($P < 0·001$) for control and phenobarbitone-induced rats.

Tissue concentrations were assayed on the fifth day (Table 1).

Table 1. *S*-Acenocoumarol tissue distribution following its 5-day intratesticular administration in control (n = 9) and phenobarbitone (n = 7)-treated rats[a].

	ng g^{-1} wet weight	
Tissue	Control	Phenobarbitone
Target testis[b]	370 ± 70	350 ± 30
Contralateral testis	26 ± 5	12 ± 1·6 ($P < 0·001$)
Liver	360 ± 30	340 ± 25
Kidney	31 ± 2	20 ± 4 ($P < 0·01$)
Observed advantage[c]	14·5 ± 1·5	29 ± 4 ($P < 0·001$)
Calculated advantage[d]	23·5	31·5

[a] Data are presented as mean ± s.d. *S*-Acenocoumarol (1 $\mu g\ h^{-1}$) was administered via an osmotic minipump. Phenobarbitone was administered continuously in the drinking water.
[b] The left testis was chosen as the target testis.
[c] The advantage of local delivery is defined as the ratio of target tissue drug concentration following local and systemic delivery, respectively (see Chen & Gross 1980). In the experiments presented it is the ratio between left and right testis.
[d] By $R_D = 1 + Cl/Q_T$ (Chen & Gross 1980), where R_D is the advantage as defined by (c), Cl is the systemic *S*-acenocoumarol clearance, and Q_T is the plasma flow through the testis (0·15 mL min^{-1}; Nishiyama et al 1976).

Liver contained 360 ± 30 ng g^{-1}, and kidneys 31 ± 2 ng g^{-1} AC. The left testis (i.e. the target testis) contained 370 ± 70 ng g^{-1} of AC, the right testis 26 ± 5 ng g^{-1}.

In the phenobarbitone-treated animals liver AC was not different from control rats, nor was the AC amount in the target testis. The amount in the contralateral testis was significantly lower as was the amount in the kidney (Table 1).

Intrarenal infusion. As can be seen in Table 2, the plasma and liver concentrations of AC after 5 days of intrarenal infusion of 1 $\mu g\ h^{-1}$ of AC were 5·5 ± 1 ng mL^{-1} and 289 ± 20 ng g^{-1},

Table 2. *S*-Acenocoumarol tissue distribution following its 5-day intra-renal (n = 6) administration[a].

Tissue	ng g^{-1} wet weight
Target kidney	30 ± 2
Contralateral kidney	33 ± 3
Liver	290 ± 20
Testes	25 ± 4
Plasma	5·5 ± 1
Observed advantage[b]	0·9 ± 0·05
Calculated advantage[c]	2

[a] *S*-Acenocoumarol (1 $\mu g\ h^{-1}$) was infused into the right kidney via an osmotic minipump.
[b] For the meaning see Table 1.
[c] For the meaning see Table 1. Plasma flow through one kidney is approximately 3·5 mL min^{-1} (Nishiyama et al 1976).

respectively. In the right kidney i.e. the target organ, 30 ± 2 ng g^{-1} of AC was found and in the left kidney $33{\cdot}6 \pm 2{\cdot}5$ ng g^{-1}. The concentration in the testes was 25 ± 4 ng g^{-1}.

Advantage of target-directed drug delivery. The regional advantage of target-directed drug delivery in our experiments is defined as the drug distribution ratio between the target and contralateral non-target organ. The data of Table 1 show an advantage of 14·5 for the intratesticular administration in control rats. In phenobarbitone-induced rats an advantage of 29 was observed. Theory predicted an advantage of 23·5 and 31·5 for control and induced rats, respectively (Table 1). Delivery to the kidney, a high flow organ, did not give an advantage over systemic administration; the observed ratio between target and non-target kidney was 0·9. Theory, however, predicted 2 (Table 2).

Discussion

The experimental approaches we applied in this study to evaluate the theoretical considerations of target directed drug delivery need some discussion. By using testis or kidney as the target organ the benefit of target-directed drug delivery can be evaluated from one experiment; i.e. the contralateral organ serves as the systemic delivered control. Because blood flow through the left and right organ is identical, the results would have been the same if the right testis instead of the left one, or the left kidney instead of the right kidney were used as the target organ. The technique of intratesticular administration was introduced to investigate a possible effect of oral anticoagulants on testicular function, i.e. on fertility. We evaluated the technique first in 5 days studies. This evaluation contained the method of introduction and fixation of the catheter and after solving this, the pharmacokinetic and biochemical analysis. The pharmacokinetic evaluation is presented herein. We did not perform morphological examinations then, but visual inspection of the testicular tissue (stereo microscopy) did not show gross deviations. One may question the technique we used for drug administration into the testis. As rat testicular arteries do not possess branches suitable to introduce a catheter for drug administration, we decided to administer AC directly in the lumen (lobuli testis?, interstitial fluid?) of the testis. The question arises whether, via this route, the intracellular drug concentrations obtained are comparable with those that would be obtained if the drug had been administered via the systemic route. Gross deviations can be expected if a diffusion barrier exists for one or the other. Unless we are dealing with systems for drug (AC) transport from plasma to the testicular cells, the high testicular AC distribution in the non-target testis (tissue to plasma ratio was about 5) suggest the absence of a blood testis barrier for AC. A consequence of the applied technique is that the interluminal AC concentration of the target testis in steady state will be high. We therefore washed the soft testicular tissue thoroughly in ice-cold saline before homogenizing it. The same procedure was followed for the contralateral testis.

According to the theory of target-directed drug delivery, the ratio of the systemic drug clearance (Cl) and the blood flow through the target organ (Q_T) determines the regional advantage (R_D) of target organ directed drug delivery over systemic delivery (Chen & Gross 1980). In the formula:

$$R_D = 1 + Cl/Q_T$$

the calculated values for R_D did not fully predict the observed ones (Tables 1, 2). Uncertainties about the exact plasma flows might be one of the reasons for the deviations between calculated and observed values. Plasma flows were taken from literature; (i) cardiac output distribution estimated via the microsphere technique (Nishiyama et al 1986) and (ii) cardiac output estimated electromagnetically (Smits et al 1982). On the whole, however, theory and experiment were in the same range.

Comparing the regional advantages observed during intratesticular and intrarenal infusion of AC demonstrates the importance of a low blood flow of the target organ for achieving high regional advantages. Direct delivery to the kidney theoretically would result in a 2-fold higher tissue concentration. However, we observed no advantage over systemic delivery. We have no explanation for this.

In summary, the results of our experiments confirm the theories on target directed drug delivery. Regional advantage by target directed drug delivery can only be obtained for a target organ with a low blood flow relative to the systemic blood clearance of the applied drug. This condition in fact excludes high flow tissues such as liver and kidney for target-directed drug delivery, unless the aim is to make use of the first-pass effects of clearance by these organs.

References

Chen, H. S. G., Gross, J. F. (1980) Cancer Treat. Rep. 64: 31–40

Daemen, M. J. A. P., Thijssen, H. H. W., Vervoort-Peters, H. T. M., Smits, J. F. M., Struyker Boudier, H. A. J. (1986) J. Pharm. Pharmacol. 38: 122–125

Eckmann, W. W., Patlak, C. S., Fenstermacher, J. D. (1974) J. Pharmacokinet. Biopharm. 2: 257–285

Hauschka, P. V., Friedman, P. A., Traverso, H. P., Gallop, P. M. (1976) Biochem. Biophys. Res. Commun. 71: 1207–1211

Nishiyama, K., Nishiyama, A., Frohlich, E. D. (1976) Am. J. Physiol. 230: 691–698

Smits, J. F. M., Coleman, T. G., Smith, T. L., Kasbergen, C. M., Van Essen, H., Struyker Boudier, H. A. J. (1982) J. Cardiovasc. Pharmacol. 4: 903–914

Smits, J. F. M., Kasbergen, C. M., Van Essen, H., Kleinjans, J. C. S., Struyker-Boudier, H. A. J. (1983) Am. J. Physiol. 244: H304–H307

Suttie, J. W. (1980) Fed. Proc. 39: 2730–2735

Thijssen, H. H. W., Baars, L. G. M., Drittij-Reijnders, M. J. (1985) Drug Metab. Dispos. 13: 593–597

Thijssen, H. H. W., Jansen, C. A. T., Drittij-Reijnders, M. J. (1986) Biochem. Pharmacol. 19: 3277–3283

Vermeer, C., Hendrix, H., Daemen, M. (1982) FEBS Lett. 147: 317–320