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pharmacokinetics
testosterone

Percutaneous Absorption of Testosterone in the Newborn Rhesus Monkey: Comparison to the Adult

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Summary

Percutaneous absorption of testosterone was determined in newborn rhesus monkeys, an animal model which is relevant to man. Mean percentage of absorptions of 4 and 40 $\mu g/cm^2$ in the newborn were, respectively, 22.5 ± 2.2 (SD) and 6.8 ± 2.1. Statistical comparisons (Student's *t*-test) of these results with those obtained with adults show no significant difference ($P > 0.05$) in skin penetration of testosterone in newborn and adult rhesus monkeys. In the newborn, the efficiency of absorption (percentage) decreased when the topical dose was increased 10-fold. However, the total compound absorbed per cm^2 area of skin actually increased from 0.9 to 2.7 μg.

With one other newborn rhesus, a topical dose of 40 $\mu g/cm^2$ was applied to the ventral forearm and the area was occluded for 24 hr. Percutaneous absorption was 14.7%, a value twice that from nonoccluded absorption.

Systemic absorption from a topical dose becomes critical in the newborn because the ratio of surface area (cm^2) to body weight (kilograms) in the newborn is 3 times that in the adult. Given equal application area of skin per newborn and adult, the systemic absorption in the newborn becomes 3 times that of the adult when based on kilograms body weight. With a different ratio of skin surface to body weight, the therapeutic ratio probably is lower in the newborn than in the adult when the compound is applied topically.

Speculation

A high percentage of a steroid compound can be absorbed through the skin of a newborn as well as an adult. The systemic availability of a drug to the newborn following topical application can be 3 times that of the adult, per unit of body weight.

Skin permeability in the newborn infant is an important concern because of the possible toxicity which could result from this route of drug delivery. Fatal poisoning in infants has resulted from topical application of phenol (2), pentachlorophenol (1), Castellani's solution (7), and hexachlorophene (9). Newborn rhesus monkeys exposed to hexachlorophene washing show brain damage where adult rhesus monkeys do not (9). These observations and others noted below have been interpreted as indicating that the newborn has an increased permeability compared to the adult. Direct skin penetration measurement in newborn rhesus monkeys should ascertain skin barrier function in the newborn and determine whether it is different from the adult. Information from this experimental animal model justify preliminary extrapolation to man since percutaneous absorption in the adult rhesus monkey appears to be similar to man (12).

MATERIALS AND METHODS

The methods used for measuring percutaneous penetration were the procedures of Feldmann and Maibach (4, 5) and Wester and Maibach (12), where absorption is quantified on the basis of the percentage of radioactivity excreted in urine after application of a known amount of the labeled compound to the skin. To correct for excretion of radioactivity by other routes and retention of radioactivity in the body, urinary excretion data obtained after dermal application of the compound are adjusted in accordance with the urinary excretion after intravenous dosage (Table 1).

The seven newborns used in this study were obtained by cesarean section. Two were obtained from exactly timed pregnancies in which their mothers were monitored for ovulation by serial laparoscopic examination. The remaining five infants were obtained from pregnancies in which mating was restricted to 5 or 6 days around the estimated day of ovulation. Thus, a reasonably exact gestational age for each infant was known at the time of surgical delivery, and this was always within 2 weeks before the estimated day of parturition.

After an approximate 2-hr recovery period the infant was placed in a small primate restraining chair (Plas-Labs), which in turn was placed in an incubator maintained at 30° (Tele-Thermometer model 73, Yellow Springs Instrument Co., Inc.). The floor of the incubator was wire mesh, under which was a tray to collect urine.

The [^{14}C]-testosterone was obtained from New England Nuclear Corporation. Testosterone was chosen as the model compound because of the extensive information available on its percutaneous absorption in man and experimental animals (4, 12, 13). The hands of the newborn monkey were secured to the sides of the metabolism chair to prevent its wiping off the applied compound. Labeled compound (4 or 40 μg) was applied in methanol to the lightly shaved ventral forearm (1-cm^2 area), the solvent quickly evaporated, and for the next 24 hr urine was collected with the infant in the chair. Then the site of application was washed with soap and water and the infant was removed from the chair and urine collection continued.

For intravenous administration, a sterile saline solution of [^{14}C]-testosterone (4 μg) was given in the saphenous or cephalic vein. Urine collection was continued as with topical application.

Urine aliquots (1.0 ml) were mixed with 10 ml PCS solubilizer (Amersham/Searle) and total radioactivity was determined by scintillation spectrometry (Mark II, Searle Analytic, Inc.) using the channels ratio method of quench correction.

Each newborn only received one topical dose, that which was applied shortly after delivery. The intravenous administration was given to three of the newborns after their topical study, when urinary radioactivity was less than 1% of the administered dose. Both male and female animals were involved.

RESULTS

Gestational ages at delivery ranged from 155-164 days, which is near full term. Body weights ranged from 450-575 g and crown rump measurements ranged from 189-216 mm, values which are normal for newborn rhesus monkeys.

Table 1. *Percutaneous absorption of testosterone in newborn rhesus monkey*

	% dose absorbed[1]	
Time, hr	4 μg/cm²	40 μg/cm²
0-24	6.3 ± 0.9	1.3 ± 0.4
24-48	8.0 ± 0.7	2.1 ± 0.8
48-72	4.3 ± 0.2	1.6 ± 0.6
72-96	2.5 ± 1.1	1.0 ± 0.1
96-120	1.4 ± 0.4	0.7 ± 0.2
Total	22.5 ± 2.2	6.8 ± 2.1
μg absorbed[2]	0.9	2.7

[1] Mean values and standard deviations of three animals. Topical values were corrected for incomplete urinary excretion with the formula

$$\% \text{ absorbed} = \frac{\% \text{ urinary radioactivity topical}}{\% \text{ urinary radioactivity i.v.}} \times 100.$$

[2] Calculated as micrograms per 1 cm² area of skin.

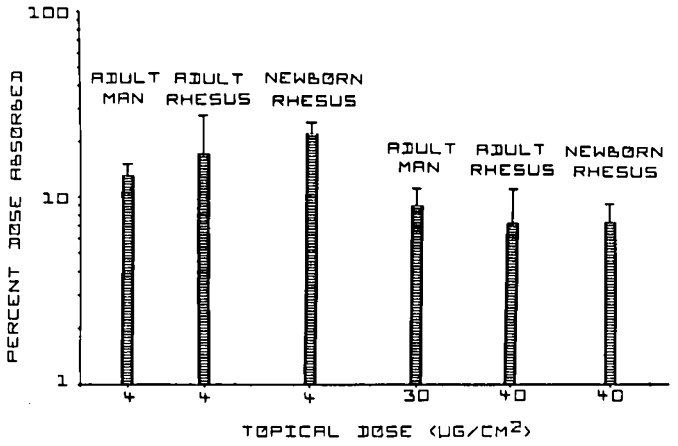

Fig. 1. Comparison of percutaneous absorption of testosterone in newborn rhesus, adult rhesus, and adult man. The black bars show the percentage of dose absorbed (and standard deviation) per topical dose (micrograms per cm²) applied. Adult rhesus and human absorption data are from References 4, 12, and 13. There is no statistically significant difference in the absorption of newborn and adult rhesus.

After intravenous administration of testosterone to three newborn rhesus monkeys, an average of 71.1 ± 9.9% (SD) of the dose was excreted in 5 days. The total excretion was used as the correction factor and subsequent absorption rates referred to are adjusted with this factor. The 5-day excretion value in adult rhesus was 79.8 ± 2.5%, (13).

Table 1 shows the percutaneous absorption of testosterone in the newborn rhesus monkey. Means and standard deviations for 24-hr time periods and 5-day total excretions are given. Additionally, micrograms absorbed per 1-cm² area of skin are given for the two dose levels. Mean percentage of absorptions of 4 and 40 μg/cm² in the newborn were, respectively, 22.5 ± 2.2 and 6.8 ± 2.1. Statistical comparisons (Student's *t*-test) of these results with those obtained with adults show no significant difference ($P > 0.05$) in skin penetration of testosterone in newborn and adult rhesus monkeys. In the newborn the efficiency of absorption (percentage) decreased when the topical dose was increased 10-fold. However, the total compound absorbed per cm² area of skin actually increased from 0.9 to 2.7 μg. Figure 1 shows a comparison of the results from the newborn rhesus with that of adult rhesus and adult human data from our published studies (12, 13).

With one other newborn rhesus, a topical dose of 40 μg/cm² was applied to the ventral forearm and the area was occluded with Saran wrap and adhesive tape for 24 hr. Percutaneous absorption was 14.7%, a value twice that from nonoccluded absorption.

DISCUSSION

When a compound is applied to the skin of a newborn, one should consider the pharmacokinetics of such an application. First, as we have shown here, a high percentage of the compound can be absorbed through the skin. Percutaneous absorption is a complex affair consisting of at least 10 separate, but interrelated processes (8). We have compared only the total dose absorbed between newborn and the adult; whether some of the steps (*i.e.*, metabolism) are different in the newborn skin remains to be explored.

Once the compound (and/or metabolites) is absorbed, it is available systemically. In the newborn the ratio of surface area (square centimeters) to body weight (kilograms) is 3 times that of the adult (11). Therefore, given equal application area of skin per newborn and adult, the systemic absorption seen in the newborn can be much more when based on kilograms of body weight. As an example, if 0.1 g of a compound is applied to the total skin surface of an adult who weighs 70 kg, and 20% of the dose is absorbed, then

Systemic availability (mg/kg)
= dose (mg) × % absorbed/body weight (kg)
= 100 mg × 0.2/70 kg = 0.28 mg/kg

The surface area of a newborn is 2,200 cm², or 13% of that of an adult (17,000 cm²). Applying the same strength compound to the total surface of a newborn would take only 13% of the 0.1 g, a topical dose of 13 mg. Given the same percutaneous absorption (20%), then in a newborn weighing 3.4 kg

Systemic availability (mg/kg) = 13 mg × 0.2/3.4 kg = 0.76 mg/kg

Therefore, by topically applying the same strength compound to both the adult and the newborn, the systemic availability in the newborn is 2.7 times that of the adult. With a different ratio of skin surface to body weight, the therapeutic ratio probably is lower in the newborn than in the adult when the compound is applied topically. This increased systemic availability in the newborn would also be interrelated with any differences in systemic metabolism between the newborn and the adult.

These results show that, at least with testosterone, skin barrier function is intact in newborn skin (as compared to the adult). Permeability in preterm infants suggests an immature barrier (6, 10), which becomes functional at full gestation. These indirect measurements included vasoconstriction, an end point determined by penetration and blood vessel reactivity, both of which may change with age. Direct measurements of permeability in the premature infant will be needed to confirm the enhanced permeability. However, with full barrier function, the newborn infant is susceptible to those factors such as occlusion, which can promote penetration and increase systemic concentration. A minimal penetrant such as hydrocortisone also showed increased systemic absorption with increased topical dose (13). Applying milligram quantities of hydrocortisone over almost the entire body surface of an infant may produce systemic effects (3).

Newborn rhesus monkeys washed with hexachlorophene developed brain effects whereas comparable 4-7-year olds did not (9). The toxic effects may not be due to increased penetration in the newborn, but from greater systemic absorption per kg body weight, or to some other pharmacokinetic parameter.

We do not wish to overinterpret the data in this animal model experiment. When techniques become available which allow the direct comparison of topical absorption in the human infant and adult, the animal model data will be found relevant or lacking. Until such experimental data are developed, these experiments provide our closest approximation of the human situation.

CONCLUSION

Percutaneous absorption of testosterone from the ventral forearm in newborn rhesus monkeys was determined as a measure of skin barrier function. Skin absorption at a concentration of 4 and 40 $\mu g/cm^2$ were, respectively, 22.5 ± 2.2% (SD) and 6.8 ± 2.1% of the applied dose, values which were not significantly different ($P > 0.05$) from those of adult rhesus and which were similar to man. If the absorption is expressed as micrograms absorbed systemically, then the amounts are 0.9 and 2.7 $\mu g/cm^2$ area of skin, a 3-fold increase in systemic absorption per 10-fold increase in topical dose. Occlusion of a dose of 40 $\mu g/cm^2$ enhanced absorption to 14.7%.

The ratio of surface area (square centimeters) to body weight (kilograms) in the newborn is 3 times that in the adult. Therefore, given equal application area of skin per newborn and adult, the systemic availability in the newborn also becomes 3-fold when based on kilograms of body weight. With a different ratio of skin surface to body weight, the therapeutic ratio probably is lower in the newborn than in the adult when the compound is applied topically. This difference between newborn and adult in systemic availability after topical application may help explain some of the toxicity reported in newborns.

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serum calcitonin

Serial Measurements of Serum Calcium, Magnesium, Parathyroid Hormone, Calcitonin, and 25-Hydroxy-Vitamin D in Premature and Term Infants during the First Week of Life

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Summary

The mean ± SEM of the cord, 48-hr, and 7-day values for serum calcium, magnesium, human calcitonin (HCT), parathyroid hormone (PTH), and 25-hydroxy-vitamin D (25-OHD) for premature and term infants can be seen in Table 1. Mean cord calcium concentrations were similar for term and premature infants. Serum calcium concentrations fell in both term and premature infants at 48 hr, but decreased more in the premature infants (from 10.23 ± 0.30 to 8.74 ± 0.19 mg/dl) than in the term infants (from 10.5 ± 0.26 to 9.6 ± 0.23 mg/dl). Serum calcium values increased from 48 hr to 7 days in both groups, and there was no significant difference between term and premature infants' serum calcium concentrations (10.6 ± 0.28 and 10.12 ± 0.3 mg/dl, respectively) at that time. There was no significant difference between term and premature cord serum magnesium concentrations. Serum magnesium concentrations increased similarly by 48 hr in both groups and remained at these concentrations at 7 days of life. Serum HCT concentrations were elevated above normal adult levels (71.9 ± 6.6 pg/ml,