PHARMACOKINETIC STUDY ON THE PERCUTANEOUS ABSORPTION OF *p*-AMINOBENZOIC ACID FROM THREE SUNSCREEN PREPARATIONS

A. ARANCIBIA - G. BORIE - E. CORNWELL - C. MEDRANO

FACULTY OF CHEMICAL AND PHARMACOLOGICAL SCIENCES - UNIVERSITY OF CHILE
CASILLA 233 SANTIAGO (CHILE)

SUMMARY. — *A urinary excretion study was conducted in six healthy male subjects to evaluate the percutaneous absorption of* p-*aminobenzoic acid (PABA) from three sunscreen preparations. The study was a crossover design and urine samples were collected during* 48 *hours after the application of the sunscreen preparations on the face, neck, trunk and upper extremities. The volunteers also received a* 500 *mg dose of PABA orally.*

No statistically significant differences in the total amount recovered in the urine were found between the three kinds of formulations, but a great intersubject variation was observed.

When PABA was given orally 50.5% *to* 83.1% *was excreted as acetylated derivative. However, when the compound was applied topically the degree of metabolization of PABA increased significantly and the level of acetylated PABA excreted fluctuated between* 69.7% *and* 90.2%.

RIASSUNTO. — *È stato studiato l'assorbimento percutaneo dell'acido* p-*aminobenzoico (PABA) contenuto in tre preparati antisolari in* 6 *soggetti sani, di sesso maschile, attraverso l'escrezione urinaria. Lo studio era di tipo « crossover » e i campioni di urina sono stati raccolti nelle* 48 *ore successive all'applicazione dei preparati sul viso, sul collo, sul tronco e sulle estremità superiori. Ai volontari è stata anche somministrata una dose di* 500 *mg di PABA.*

Non si è osservata alcuna differenza statisticamente significativa nel quantitativo totale dei tre preparati presente nelle urine, mentre si è notata una notevole variazione intersoggettiva.

In seguito a somministrazione orale di PABA è stato osservato che il 50,5-83,1% *di esso è stato eliminato come derivato acetilato mentre con l'applicazione topica il grado di metabolizzazione del PABA è aumentato significativamente e il livello di PABA acetilato eliminato è risultato essere compreso tra* 69,7% *e* 90,2%.

Sunscreens are used as a protective measure to prevent sunburn and avoid sun injury. In many countries this kind of preparations is considered a cosmetic. Certain major characteristics that an ideal sunscreen should possess have been outlined. These are: a) high protection against sunburning anywhere on the earth's surface, b) maintenance of significant protection under conditions of normal usage, c) cosmetic elegance, d) stability and e) lack of toxicity (1, 2).

When cosmetics or topical dosage forms are applied they are intended to act on the skin and the active substances they contain should not be absorbed into the systemic circulation. During the last few years there has been an increasing interest on the safety of cosmetic products. Attention has been focused on their toxicological and sensitizing properties as well as on their effect on the skin. Nevertheless, few works have so far been devoted to their percutaneous absorption and to the extent of such absorption when cosmetics containing active substances are applied to the skin.

We have considered of interest to study the percutaneous absorption of a chemical agent incorporated into sunscreen preparations. The normal conditions under which a sunscreen is employed may favor the absorption of the active substances. Such conditions are as follows: application over an extensive area of the skin; exposure to the sun, which raises the temperature of the skin and generates sudoration; repetitive application of the product; oily or emulsioned vehicle which may favor hydration and penetration; high concentration of the active ingredient, etc. Para-aminobenzoic acid (PABA) and its esters are the most popular substances employed as sunscreens (3, 4, 5). The present work deals with the absorption and disposition kinetics of PABA after topical applications of three different sunscreen preparations on the skin of healthy volunteers. PABA and its main metabolites were determined in the urine.

Experimental

Sunscreen preparations

Three formulations containing 5% of PABA were employed: A) Hydroalcoholic gel containing methylcellulose; B) Anionic O/W emulsion at pH 4.2 in which PABA is mainly suspended; C) Anionic O/W emulsion at pH 6.5 in which the active ingredient is dissolved.

Subjects

Six apparently healthy male volunteers participated in the study. They ranged in age from 31 to 57 years (mean 42.3 ± 9.6) and in weight from 62 to 82 kg (mean 70.8 ± 8.2). The subjects were divided into three groups of two subjects each. Upon getting up in the morning, each subject received

topically 20 g of preparation on the face, neck, trunk and upper extremities. All of them took part in three experiments one week apart. Urine was collected immediately before the application of the sunscreen preparation and 2, 4, 8, 12, 16, 24, 28, 32, 36, 40 and 48 hours thereafter. The volume of each sample was measured and an aliquot was frozen until analyzed. Assays were performed within 72 hours. All volunteers were asked to abstain from alcoholic beverages for the 48 hours preceeding each experiment. They also took no drugs for at least one week before each trial. All the subjects were informed about the nature of the experiments and signed a consent form prior to the study. One week before starting with the topical experiments, each subject received a 500 mg oral dose of PABA dissolved in 8 ml of ethanol and 150 ml of water. Urine was collected before dosage and at 1, 2, 4, 6, 8, 10 and 24 hours thereafter, and treated as mentioned above.

Experimental design

The sequence of the topical applications was established according to the crossover matrix described in Table I.

TABLE I

Experimental design.

Group	Subject	Time periods		
		I	II	III
G1	1 and 2	A	B	C
G2	3 and 4	B	C	A
G3	5 and 6	C	A	B

Assay of urine samples

PABA in the urine was determined by the method of Bratton and Marshall (6). This assay measures PABA together with its metabolite para-amino hippuric acid (PAHA). The total amount of PABA plus metabolites, mainly PAHA and acetylated PABA, was measured by hydrolysis of 5 ml of urine with 0.25 ml of 4 N HCl for 2 hours at 95°, followed by the copulation reaction with N-1 naphthyl ethylendiamine. The difference between "free" PABA compounds determined before hydrolysis and "total" PABA determined after hydrolysis, was used to express the amount of "acetylated" PABA compounds (7).

Statistical analysis

The results were analyzed according to a three way crossover analysis of variance (8).

Results and discussion

After oral administration, the elimination of PABA from the body assessed by measuring urine excretion was very rapid. Maximum urinary excretion rates were reached within 1 to 2 hours. Six hours after the administration about 90-95% of the total amount excreted in 24 hours had been eliminated. This was 334.7 mg ± 47.7 and represented 66.9% of the administered dose.

Semilogarithmic plots of the excretion rates of total PABA and acetylated PABA give straight lines. Fig. 1 shows the elimination profile

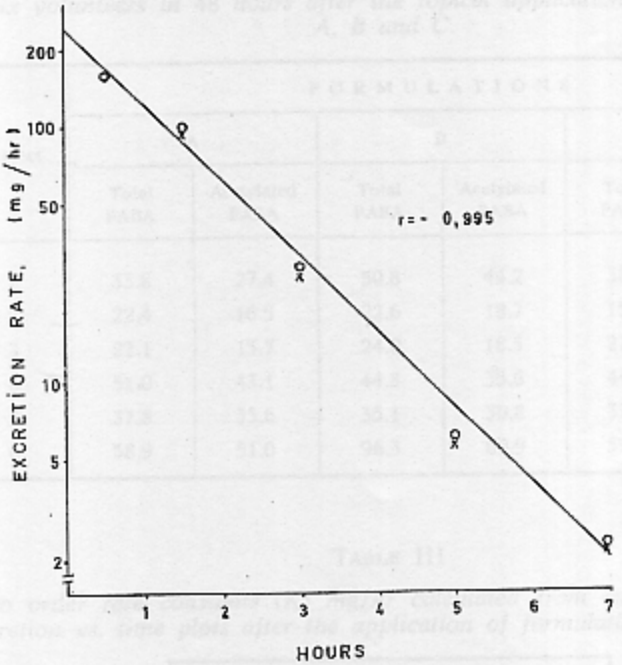

Fig. 1

Urinary excretion rate of total PABA (○) after oral administration of a hydro-alcoholic solution containing 500 mg of PABA. (×) points correspond to the acetylated derivative.

of total and acetylated PABA obtained in one of the volunteers. The mean elimination rate constant determined for all the subjects participating in the oral study was 0.746 ± 0.165 hr^{-1} which corresponds to a half life of 55.7 min. The results of the urinary excretion study after topical applications of the three sunscreen preparations are summarized

in Table II. The data were also plotted as cumulative amounts of total PABA and acetylated PABA versus time. Values increase in direct proportion to time. The slope of these lines is the excretion rate of the compound and, the percutaneous absorption being the rate-limiting step, the slopes represent the zero order rate constant for the absorption process (9, 10, 11). Table III contains the calculated zero order rate constants. Fig. 2 shows two typical examples.

TABLE II

Cumulative urinary excretion of acetylated PABA and total PABA (mg) in six volunteers in 48 hours after the topical application of formulations A, B and C.

Subject	FORMULATIONS					
	A		B		C	
	Total PABA	Acetylated PABA	Total PABA	Acetylated PABA	Total PABA	Acetylated PABA
1	33.8	27.4	50.8	44.2	38.7	32.8
2	22.4	16.5	22.6	18.7	15.8	10.6
3	22.1	13.7	24.0	18.5	27.3	18.3
4	51.0	43.1	44.5	35.6	44.7	38.1
5	37.8	33.6	35.1	30.8	37.8	32.6
6	58.9	51.0	96.3	89.9	59.6	54.0

TABLE III

Zero order rate constants (K) mg/hr calculated from cumulative urinary excretion vs. time plots after the application of formulations A, B and C.

Subject	FORMULATION		
	A	B	C
1	0.744	1.182	0.866
2	0.483	0.466	0.304
3	0.421	0.550	0.484
4	1.143	0.394	0.917
5	0.805	0.756	0.881
6	1.340	2.215	1.362

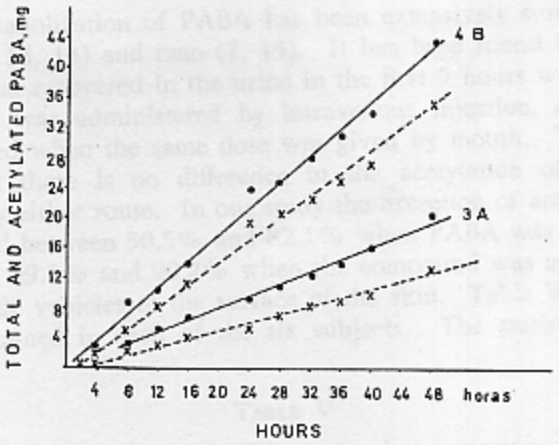

Fig. 2

Cumulative urinary excretion of total PABA (O) and acetylated PABA (×) after topical application of 20 g of the formulation A on subject 3 and of the formulation B on subject 4.

No statistically significant differences in the amounts of PABA excreted in the urine were found after application of the three sunscreen preparations. On the other hand, a great intersubject variation was observed. Table IV summarizes the analysis of variance of the urinary excretion of total PABA in the six subjects after the three treatments.

TABLE IV

Analysis of variance of urinary excretion of total PABA after the three treatments.

Source of variation	Degrees of freedom	Sum of squares	Mean square	F	Significance level
TOTAL	17	6,200.15		—	—
Subjects	5	5,055.65	1,011.13	10.79	$p < 0.0025$
Groups	2	1,854.77	927.39	9.90	$p < 0.01$
Subjects in Groups	3	3,200.88	1,066.96	11.39	$p < 0.005$
Sequence	2	134.67	67.34	0.72	N.S. $p > 0.25$
Formulation	2	260.11	130.06	1.39	N.S. $p > 0.25$
Residue	8	749.72	93.70	—	—

The metabolization of PABA has been extensively studied in both animals (12, 13, 14) and man (7, 15). It has been found that 45-51% of the material recovered in the urine in the first 5 hours was acetylated when PABA was administered by intravenous injection, and 45-53% was acetylated when the same dose was given by mouth. These results indicate that there is no difference in the acetylation of PABA administered by either route. In our study the excretion of acetylated compound ranged between 50.5% and 82.1% when PABA was given orally, and between 69.7% and 90.2% when the compound was applied in the dermocosmetic vehicles to the surface of the skin. Table V summarizes the data obtained in each of the six subjects. The statistical analysis

TABLE V

Urinary excretion of acetylated PABA expressed as percentage of total PABA after oral and topical administration.

Subject	Topical application				Oral administration
	FORMULATION				
	A	B	C	mean ± S.D.	
1	81.1	87.2	84.6	84.3 ± 3.1	71.4
2	73.6	82.9	67.0	74.5 ± 8.0	82.1
3	64.9	77.1	67.0	69.7 ± 6.5	56.4
4	82.9	80.0	85.2	82.7 ± 2.6	50.5
5	88.9	87.6	86.2	87.6 ± 1.4	67.2
6	86.6	93.4	90.6	90.2 ± 3.4	76.6

using the paired Student's "t" test indicates that the differences are significant ($P < 0.05$). The finding is interesting from a pharmacokinetic point of view in that it indicates that when the substance reaches the circulation through the skin, the input occurs very much like a zero order kinetics and the slow and sustained presentation of the compound to the metabolizing enzymes results in a more complete acetylation. The situation is similar to that observed by Drucker *et al.* when PABA was administered in an intravenous infusion or given by mouth divided into small doses every 30 minutes. In both cases the acetylated compound recovered in the 5-hours urine was 91-94% (7).

The impermeability of skin is associated with its functions as a protective barrier against invasion by microorganisms, viruses, and toxic chemicals as well as the egress of physiologically essential substances,

such as water. Research on percutaneous absorption during the last few years has contributed to a better understanding of the nature and origin of the barrier properties of the skin and of the physicochemical factors governing the transdermal absorption of substances, and have opened up new approaches to using this organ as a route for administering drugs to the systemic circulation. These findings have indicated that it is feasible to administer drugs topically in such a manner that the drug content of the skin is minimized and maximum transdermal permeation of drug is attained (10, 16, 17).

The application of sunscreen preparations or other cosmetic products over an extensive surface of the skin raises the problem of absorption of chemicals into the systemic circulation. In our study we have found that the amount of PABA recovered in the urine in 48 hours fluctuated between 15.8 and 96.3 mg, which corresponds to 1.6 and 9.6% of the applied PABA. These quantities do not represent a high percentage of the amount employed in the experiments. However one could expect a greater amount to be absorbed when these products are reapplied several times and used in the ordinary way and circumstances, where favourable conditions for absorption do exist. There is general agreement on the idea that all materials applied to the human skin should be tested for both topical and general toxicity. All these substances must prove to be safe. As far as we have reviewed in the scientific literature, little attention has been devoted to studying the transdermal absorption of substances applied to the skin in sunscreen preparations. The entrance of such substance into the systemic circulation can cause deleterious effects by itself or may interact with drugs administered either previously or simultaneously with the use of the sunscreen. It has been reported that PABA diminishes the pharmacological activity of sulfonamides by interfering with their mechanism of action and decreases the effect of pyrimethamine (18).

PABA is mainly eliminated as an acetylated derivative. This metabolic way of elimination is common to many drugs, so it is possible to expect interactions between PABA and these drugs, especially if it is considered that acetylation of PABA appears to be a saturable metabolic process (7, 19). These interactions may be of clinical importance and since the sunscreens and other cosmetics containing pharmacological active substances are freely applied, we consider that their possible percutaneous absorption should be tested when a program meant to determine the safety of cosmetics is outlined.

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