Milk−Blood Transfer of ^{14}C-Tagged Polycyclic Aromatic Hydrocarbons (PAHs) in Pigs

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Polycyclic aromatic hydrocarbons (PAHs) are lipophilic organic pollutants occurring widely in the terrestrial environment. To study the transfer of PAHs in the food chain, pigs have been fed with milk spiked either with [^{14}C]phenanthrene or with [^{14}C]benzo[*a*]pyrene. The analysis of blood radioactivity showed that both PAHs were absorbed with a maximum concentration at 5−6 h after milk ingestion, similar to fat metabolism. The blood radioactivity then decreased to reach background levels 24 h after milk ingestion. Furthermore, the blood radioactivity was higher for phenanthrene (even if the injected load was the lowest) than for benzo[*a*]pyrene, in agreement with their solubility difference. These findings suggest that milk fat and PAHs were absorbed during the same time period.

Keywords: *PAHs; milk; blood; animal; pig; transfer; toxicity; bioavailability*

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are organic contaminants widely occurring at trace levels in ecosystems such as soils, sediments, the atmosphere, and plants (*1*−*5*). Although PAHs have natural sources, for example, vegetation fires, soil records have shown that PAH levels have increased after the start of industrial activities (*6*). The occurrence of point-source pollution such as oil spills, ancient industrial sites (*7*), sewage sludges (*8*), and vehicle exhausts from urban areas and highways has raised concern about the possible transfer of PAHs from plants to dairy food and then to living organisms. Nonetheless, reports on PAH occurrence in dairy food and animals are scarce (*9, 10*). Moreover, food-animal transfer pathways of PAHs are so far poorly known due to the absence of investigations involving tracers. Here, we report a study of milk−blood transfer of PAHs using two ^{14}C-tagged compounds, the three-ring phenanthrene of moderate water solubility (1.2 mg/L) and the five-ring benzo[*a*]pyrene of low water solubility (3.8 μg/L).

MATERIALS AND METHODS

Spiked Milk. Radioactivity handling and animal tests were performed in accordance with French policies. One liter of milk was spiked with 50 μCi of [7,10-^{14}C]benzo[*a*]pyrene (54 mCi/mmol, Amersham) in 1 mL of toluene. One liter of milk was spiked with 15 μCi of [9-^{14}C]phenanthrene (55 mCi/mmol, Amersham) in 1 mL of ethanol.

Animals and Diets. The animal protocol was in accordance with the general guidelines of the Council of European Communities (*11*). Two castrated Large White pigs (body weight = 48 kg) from the herd of a commercial farm were used. The pigs were fed twice daily during one week in our laboratory with a well-balanced diet (800 g/meal) based on wheat and soybean to meet the maintenance and growing needs of the animals as prescribed by Henry et al. (*12*). Each animal was fitted with two catheters, one in the portal vein and one in the brachiocephalic artery. Anesthesia was induced with sodium thiopentone (10−15 mg/kg) and maintained with fluothane inhalation (0.5−1.5% as required). The animals were fitted with a cuffed endotracheal tube, and the lungs were mechanically ventilated at a minute volume of 150 mL/kg. Surgery was performed under strictly aseptic conditions. The animals began to eat the day after the operation and rapidly recovered to assume their normal growth rate (400 g/day). To prevent obstruction by blood clots, the cannulas were rinsed daily with a heparinized (100 IU/mL) NaCl solution (9 g/L). This was achieved under aseptic conditions to avoid any risk of infection. The ^{14}C spike experiment began once pigs had completely recovered from surgery (>5−6 days). Throughout the experimental period, they were kept in individual cages allowing easy access to the cannulas for blood sampling in the portal vein and in the brachiocephalic artery.

^{14}C-Labeled PAH Experiment. Fourteen days after surgery, 1 L of either [^{14}C]benzo[*a*]pyrene- or [^{14}C]phenanthrene-

Table 1. ^{14}C Radioactivity Level in Portal and Arterial Blood after Ingestion of [^{14}C] Benzo[*a*]pyrene or [^{14}C]Phenanthrene by the Growing Pig

h after ingestion	arterial blood, Bq/mL	portal blood, Bq/mL
	Benzo[*a*]pyrene	
0	0 ± 0.00	0 ± 0.00
1	1.13 ± 0.02	1.78 ± 0.01
2	2.82 ± 0.11	3.22 ± 0.15
3	2.61 ± 0.01	4.45 ± 0.07
4	3.81 ± 0.10	5.12 ± 0.24
5	4.96 ± 0.16	6.98 ± 0.11
6	5.73 ± 0.01	7.47 ± 0.28
9	4.04 ± 0.06	4.50 ± 0.01
24	1.49 ± 0.01	1.65 ± 0.00
	Phenanthrene	
0	0 ± 0	0 ± 0
1	12.89 ± 0.01	15.32 ± 0.08
2	18.06 ± 0.67	19.58 ± 0.09
3	22.70 ± 0.22	23.08 ± 0.26
4	24.35 ± 0.25	26.00 ± 0.25
5	25.63 ± 0.95	26.69 ± 0.03
6	22.57 ± 0.39	23.55 ± 0.16
9	12.03 ± 0.38	11.72 ± 0.23
24	2.60 ± 0.06	3.25 ± 0.05

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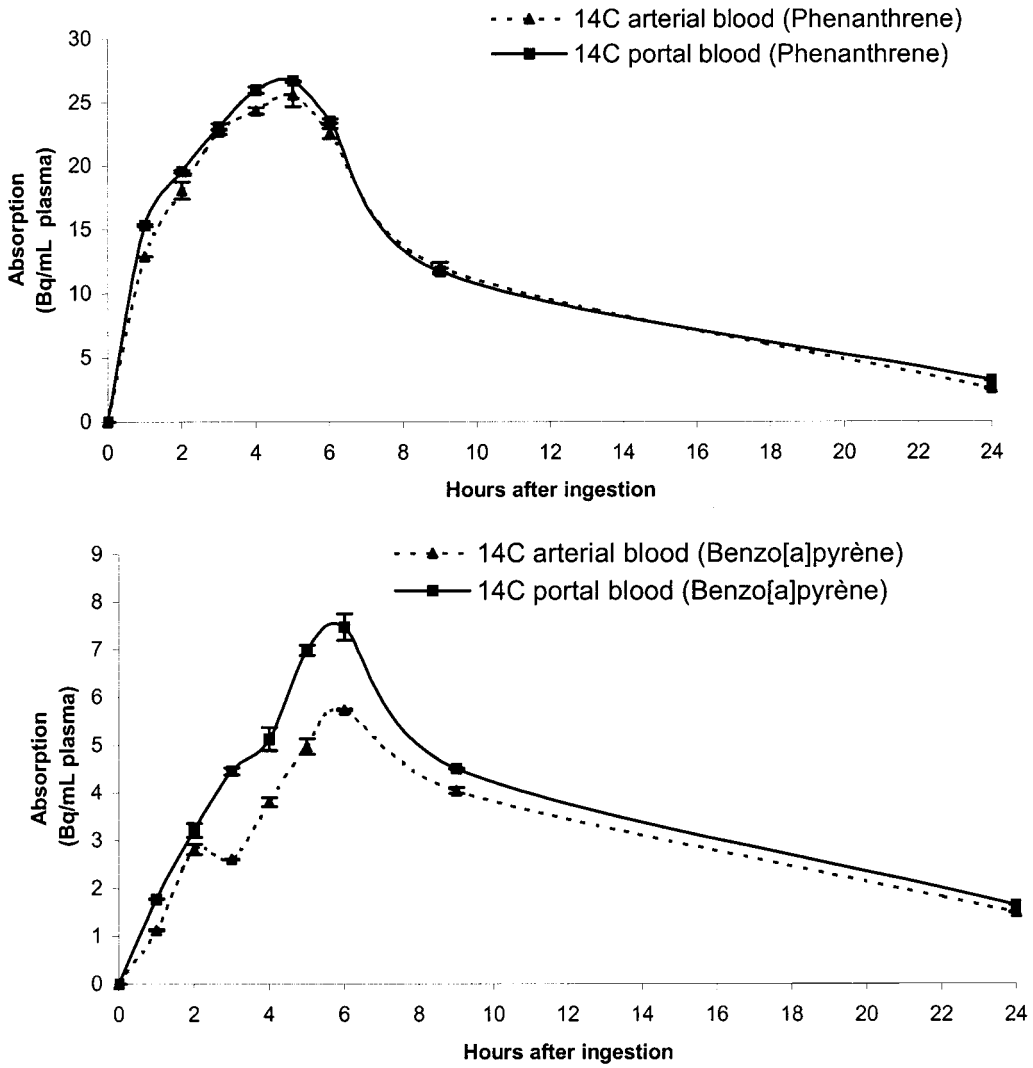

Figure 1. Portal and arterial kinetics of ^{14}C after ingestion by the growing pig of 1 L of milk spiked with [^{14}C]benzo[*a*]pyrene or [^{14}C]phenanthrene.

Figure 2. Porto-arterial differences of ^{14}C after ingestion by the growing pig of 1 L of milk spiked with [^{14}C]benzo[*a*]pyrene or [^{14}C]phenanthrene.

spiked milk was fed to the animals. Ten-milliliter portal and arterial blood samples were then collected simultaneously (1) prior to the milk distribution and (2) 1, 2, 3, 4, 5, 6, 9, and 24 h after milk ingestion. Blood samples were immediately centrifuged for 10 min at 3000*g* (4 °C). Plasma supernatant was then collected and stored at −20 °C. ^{14}C in plasma was

measured by direct counting (10 min) of duplicate 1 mL samples in 10 mL of Ultimagold scintillation fluid (Beckman) using a Tricarb 460 CD liquid scintillation counter (Packard). Radioactivity is expressed in becquerels per milliliter of plasma.

RESULTS AND DISCUSSION

Pigs were fed with milk spiked either with [^{14}C]-phenanthrene or with [^{14}C]benzo[*a*]pyrene. To study the kinetics of PAH transfer, blood was sampled in the portal vein and in the brachiocephalic artery over a 24-h period. Values of blood plasma radioactivity are reported in Table 1. Several noteworthy observations were made. First, the radioactivity was readily observed 1 h after milk ingestion (Figures 1 and 2). It increased rapidly to a maximum at ~5−6 h and then decreased to reach background levels after 24 h. Second, the blood plasma radioactivity was higher at peak maximum for phenanthrene than for benzo[*a*]pyrene. It is interesting to note that the radioactivity level from the phenanthrene was ~3 times lower than that of benzo[*a*]pyrene. Third, although radioactivity levels were slightly higher in the portal vein compared to the brachiocephalic artery, the absorption kinetics were similar (no time shift). These findings have several implications.

The radioactivity peaks observed after 4−6 h of PAH ingestion were in the same range of those observed for milk fat absorption (*13*) and differed notably from peak absorption of glucose (45 min) and protein (30 min) (*14*). This result thus suggested that PAH absorption was most likely related to fat absorption. This behavior was supported by the fact that phenanthrene and benzo[*a*]-pyrene kinetics of absorption were similar, notably with the absence of time shift (Figures 1 and 2). Indeed, one would expect a much faster transfer of phenanthrene in blood because the water solubility of phenanthrene (1.2 mg/L) was much higher than that of benzo[*a*]pyrene (3.8 μg/L). On the contrary, because both absorption curves were similar, it can be concluded that both compounds were not transferred in the aqueous phase, thus favoring the lipid phase as transport means. Finally, PAH absorption, related to fat absorption, may be in agreement with the high lipophilicity of phenanthrene (log K_{ow} = 4.5) and benzo[*a*]pyrene (log K_{ow} = 6.3) and with the behavior of other lipophilic contaminants such as dioxins (*15*−*19*). On these grounds, it can be sugested that PAHs and fat milk were absorbed during the same time period. Nonetheless, other transfer mechanisms cannot be excluded, notably because this study has not taken into account the radioactivity transferred to animal compartments other than blood plasma.

For each compound, the radioactivity level in blood plasma was higher for the portal vein than for the brachiocephalic artery (Figures 1 and 2). This finding suggests a preferential transfer of PAHs by the blood pathway. Indeed, there are two main pathways of nutrient absorption through guts: (1) the blood pathway, which involves direct transfer of blood into the portal vein, and (2) the slower blood transfer by the lymphatic pathway, which can be seen partly at the brachiocephalic artery. Alternatively, the lower radioactivity level observed in the brachiocephalic artery can be explained by rapid absorption of PAHs in the liver and other tissues. Whatever pathway, our study has clearly shown that PAHs and milk fat were absorbed during the same time period.

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