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RECENT ADVANCES IN NONINVASIVE SYSTEMIC DELIVERY OF PHARMACEUTICALS AND BIOPHARMACEUTICALS

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ABSTRACT

Many systemically-active pharmaceuticals and biopharmaceuticals are reportedly subjected to extensive pre-systemic elimination when taken orally.

To circumvent this dilemma in systemic delivery, extensive research efforts have recently been devoted to explore various nonparenteral (noninvasive) routes of administration for enhancing systemic bioavailability of pharmaceuticals and biopharmaceuticals, which have been reportedly subjected to extensive pre-systemic elimination, via the bypassing of hepato-gastrointestinal "first-pass" metabolism. Using nicotine, a pharmaceutical, and enkephalin, a biopharmaceutical, the systemic delivery of pharmaceuticals and biopharmaceuticals through the skin and the various absorptive mucosae has been illustrated. The mechanisms and kinetic processes involved for their systemic delivery are explored and various technical issues encountered are discussed in this article.

INTRODUCTION

Since the inception of treating illness with medication, the oral route has gained popularity as the most prescribed route for the systemic delivery

of pharmaceuticals and over 85% of the pharmaceutical products have been marketed in oral dosage forms.

However, it has been increasingly learned that when administered by the oral route, many systemically-active pharmaceuticals are subjected to extensive pre-systemic elimination by gastrointestinal degradation and/or hepatic metabolism before they reach the target tissue(s), via the systemic circulation (Figure 1). Results of low systemic bioavailability, short duration of therapeutic activities and/or formation of less active or inactive or even toxic metabolite(s) have been often reported. This dilemma is even more serious for the systemic delivery of biopharmaceuticals, produced either naturally in the body or commercially by biotechnology processes.

Delivery of these pharmaceuticals and biopharmaceuticals via the skin, which envelops the body surface, or the absorptive mucosa, which covers various externally-accessible body cavities, has been learned to have the potential advantage of bypassing the hepato-gastrointestinal "first-pass" elimination associated with oral administration (Figure 2). These nonparenteral routes of systemic delivery are noninvasive in nature and can provide both the benefits of direct entry of systemically-active therapeutic agents into the systemic circulation, like a parenteral route of administration but without its health hazards, as well as maintenance of a steady, prolonged and therapeutically-effective level, which duplicates a closely-monitored intravenous infusion [1].

In this article, the systemic delivery of pharmaceuticals and biopharmaceuticals that have been reportedly subjected to extensive pre-systemic elimination when administered orally, through the skin and/or the absorptive mucosae will be discussed. Several recent developments in the field will also be presented as the examples for illustration.

NONINVASIVE SYSTEMIC DELIVERY OF PHARMACEUTICALS

In response to the marketing success of nitroglycerin-releasing transdermal delivery devices, several transdermal drug delivery (TDD) systems

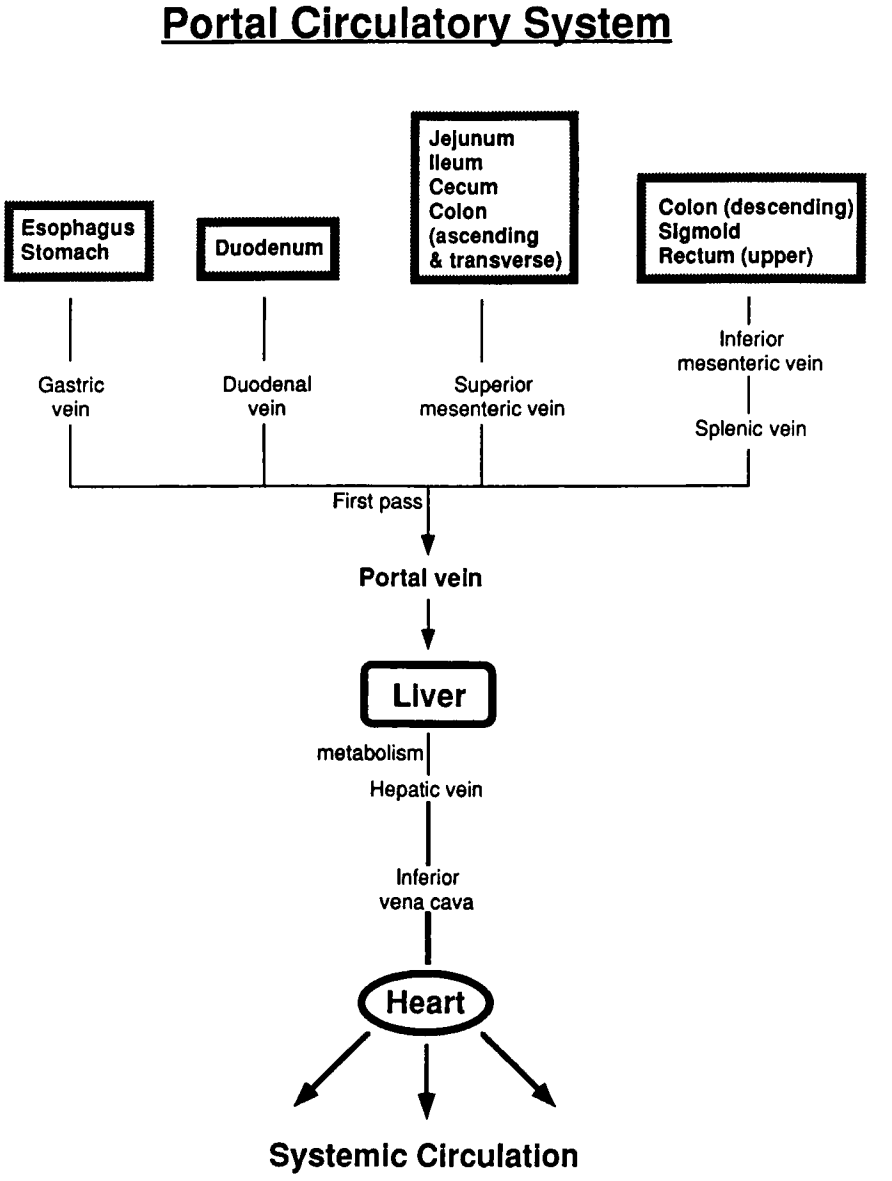

FIGURE 1

The various circulatory pathways that a drug molecule takes, following its absorption from the various segments of gastrointestinal tract, before it reaches the systemic circulation.

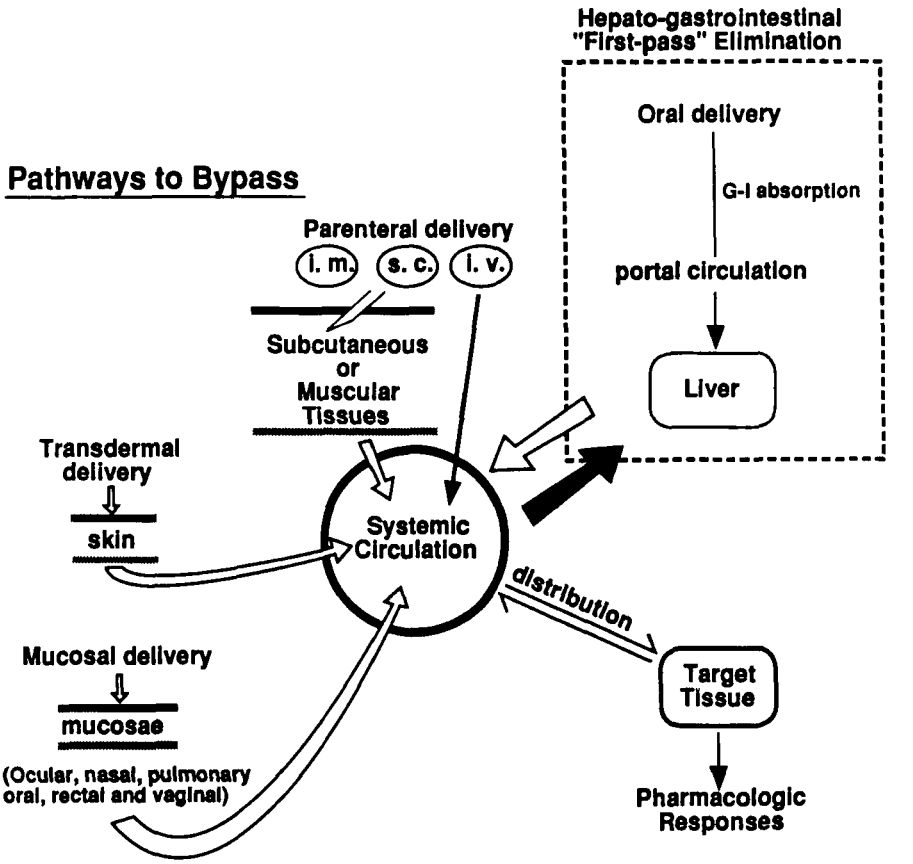

FIGURE 2

The various parenteral and non-parenteral routes of administration as the potential pathways for the systemic delivery of drug molecule via the bypass of hepato-gastrointestinal "first-pass" elimination associated with oral administration.

have been developed for the systemic delivery of various organic-based pharmaceuticals with high hepatic clearance, such as isosorbide dinitrate, the synthetic organic nitrate for the prophylaxis and treatment of angina pectoris, and estradiol-17β, the endogenous estrogenic hormone for the medication of postmenopausal syndromes, just to name a few. The most recent development of transdermal delivery systems for nicotine, which has been

reported to be metabolized extensively with a systemic bioavailability of only 7.6 (±3.1)% when taken orally, to assist smokers to quit smoking could serve as an interesting case.

Health Hazards of Smoking

Cigarette smoking has been identified as one of the most significant causes of death and disease [2]. In the United States alone, more than 1 in 6 deaths has been found to be associated with smoking, representing 30% of all cancer deaths (including 87% of lung cancer-related deaths), 21% of deaths from coronary heart disease, 18% of stroke deaths and 82% of deaths from chronic obstructive pulmonary disease [3]. Another 53,000 deaths in the United States are attributed annually to the effects of passive smoking [4].

It has been estimated that by the year 2000, approximately 3 million smoking-related deaths, in the United States alone, could have been avoided or postponed by giving up or not having started smoking in the period 1965-85 [3].

Knowing of these consequences has prompted the introduction of the various anti-smoking campaigns and smoking cessation therapies.

Composition of Smoking Tobacco

Approximately 4,000 chemical compounds are generated when tobacco is burning, and the smoke consists of gaseous and particulate phases [5]. The gaseous phase contains many gaseous compounds that produce undesirable effects, like carbon monoxide, and some of them are potent inhibitors of ciliary movement, while the particulate phase contains nicotine, tar and water. Tar consists primarily of polycyclic aromatic hydrocarbons, some of which are documented carcinogens, such as nonvolatile nitrosamines and benzo[a]pyrene. The components most likely to contribute to the health hazards of smoking are carbon monoxide, nicotine and tar.

The composition of the actual smoke delivered to a smoker depends not only on the composition of the tobacco, but also on the packing characters

of the cigarette. When a cigarette is smoked, using a standard smoking machine, approximately 0.5-35 mg of tar and 0.05-2.0 mg of nicotine are delivered. The actual content of nicotine in tobacco varies in the range of 0.2-5%, but it is in the range of 1-2% for smoking tobaccos.

Chronic Toxicity of Tobacco Smoking

Chronic use of tobacco is causally linked to a variety of serious diseases, ranging from coronary artery disease to lung cancer. The likelihood of developing any one of these disorders increases with the degree of exposure. All male smokers, for example, the overall mortality ratio is about 1.7 compared with non-smokers. The ratio increases to 2.0 for those smoking two packs a day, and it is even higher among inhalers than non-inhalers. For women, smoking a little over a pack a day is associated with a 5-fold increase in the fatal coronary heart disease [6]. There are some differences in mortality rate among cigarette, cigar and pipe smokers, which are probably related to the extent of inhalation and the degree of exposure to all the constituents of smoke.

Smoking has been reported to also affect the metabolism of pharmaceuticals. A wide variety of pharmaceuticals were found to be metabolized more rapidly in smokers than in non-smokers, probably as a result of the induction of enzymes in the intestinal mucosa or in the liver by components of tobacco smoke, and higher doses may be required for the treatment. However, enzymatic activities begin to return toward baseline within a few days of smoking cessation.

The likelihood of developing a smoking-related disorder has been reported to be reduced by smoking cessation. Over a period of 5-10 years, the risk falls to a level only slightly above that of the non-smoker. Even though the destruction of lung tissue is not reversible with the cessation of smoking, however, the rate of decline in the pulmonary functions of the smokers begins to resemble that of the non-smokers.

Pharmacokinetics of Nicotine from Smoking

Nicotine is readily absorbed from the respiratory tract, buccal mucosa and skin [7]. It has a systemic bioavailability of 90% via smoking (in habitual smokers) and of only 30% by oral administration. It has a volume of distribution of 2.6 (±0.9) l/kg and eliminates with a half-life of 2.0 (±0.7) hrs and a clearance of 18.5 (±5.4) ml/min.kg

The nicotine, which is suspended on minute particles of "tar" in cigarette smoke, is quickly absorbed from the lung, almost with the same efficiency as that of i.v. injection. It reaches the brain within 8 seconds after inhalation. Nicotine has two pka's: one at 7.84 and another at 3.04; thus in cigarette smoke, which is somewhat acidic, nicotine is not well absorbed from the oral mucosa; pipe and cigar smoke, which is more alkaline (pH 8.5), nicotine is better absorbed. The peak plasma concentrations of nicotine, after a cigarette is smoked, range typically 25-50 ng/ml[8]. A typical plasma profile of nicotine following the hourly smoking of cigarette is shown in Figure 3.

The plasma profile indicates that the elimination of nicotine is multi-exponential. Following the smoking of a single cigarette for 10 min, plasma concentration of nicotine reaches the peak level within 10 min and then declines rapidly with a half-life of approximately 30 min, primarily reflecting the distribution of nicotine. After long-term smoking, the elimination half-life is prolonged to 2 hrs. For the average smoker, plasma concentrations of nicotine are somewhat higher at the end of the day. Nicotine is oxidized to its major metabolite, cotinine, which is eliminated more slowly (with a half-life of ~19 hrs). Thus, cotinine is a better measure of overall intake than nicotine itself [8].

Approximately 80-90% of nicotine is altered in the body, mainly in the liver but also in the kidney and lung. A significant fraction of inhaled nicotine is metabolized by the lung [9]. The major metabolites are cotinine and nicotine-1'-N-oxide. Smokers appear to metabolize nicotine more rapidly than do non-smokers.

Pharmacology of Nicotine

Nicotine, which is a tertiary amine obtained from the tobacco plant, binds stereoselectively to acetylcholine receptors at the autonomic ganglia, in the adrenal medulla, at neuro-muscular junctions, and in the brain. Of its two steroisomers, S(-)-nicotine is the more active form.

Two types of CNS effects are believed to be the basis of nicotine's positively-reinforcing properties: A stimulating effect and a "reward" effect. The stimulating effect, which exerts mainly in the cortex, produces an increase in alertness and cognitive performance. On the other hand, the "reward" effect is produced via the "pleasure system" in the brain. At low doses, the stimulating effect predominates, while at high doses, the "reward" effect becomes predominant.

Intermittent i.v. administration of nicotine activates neuro-hormonal pathways, releasing acetylcholine, norepinephrine, dopamine, serotonin, vasopressin, ß-endorphine, growth hormone and ACTH.

There is considerable evidence that nicotine is the agent in tobacco responsible for the development of addiction to cigarette smoking. Smoking meets several of the standard criteria for drug addiction [3, 10]. The American Psychiatric Association has described nicotine addiction as a psychoactive substance-induced organic mental disorder.

Abstinence from or reduction in the use of cigarette smoking results in a withdrawal syndrome. The withdrawal syndrome associated with the cessation of smoking is characterized by a series of unpleasant symptoms, including psychological symptoms (such as inability to concentrate, irritability, craving for cigarette and aggressiveness) and physiological symptoms (such as headache, dizziness, sweating and constipation) [11]. These symptoms are frequently quoted as the primary reason for the high relapse rate among the smokers attempting to stop smoking [12].

Development of Tolerance, Dependence and Relapse

Tolerance develops to some of the effects of nicotine. It is likely that tolerance is due primarily to pharmacodynamic changes rather than to the alterations in pharmacokinetics of nicotine.

In human smokers, some aspects of tolerance wax and wane rapidly. Following the overnight non-smoking period, the first cigarette of the day produces a much greater cardiovascular and subjective responses than do those that follow.

Intravenous Pharmacokinetics of Nicotine

Following intravenous administration, nicotine produces a pharmacokinetic profile similar to that from smoking, which has an elimination half-life of 1-2 hours and a volume of distribution of 2-3 L/kg. Liver was found to be the major eliminating organ, with an average plasma clearance of approximately 1.2 L/min; kidney and lung were also reported to metabolize nicotine, while there is no significant metabolism occurring in the skin. More than 20 metabolites have been identified, all of them are believed to be less active than nicotine. Cotinine, which is the primary metabolite in plasma, was found to have a longer half-life (15-20 hours) and attained a higher plasma level (by 10 folds) than nicotine.

The primary metabolites detected in the urinary excretion are cotinine (15% of the dose) and trans-3-hydroxycotinine (45% of the dose). Only about 10% of nicotine dose is excreted in intact form in the urine, with high urine flow rates and urinary pH below 5. On the other hand, as much as 30% of nicotine dose may be excreted unchanged in the urine.

Transdermal and Transmucosal Permeation of Nicotine

It has been known for over 60 years that nicotine passes readily across the oral mucosa [13], but only recently has there been any attempt to examine

the process quantitatively [14-17]. Most studies of oral absorption have been concerned with nicotine derived from tobacco smoke [18].

Nicotine was found to be absorbed rapidly across the nasal mucosa when smokeless tobacco snuff was placed in the nasal cavity, which has resulted in the highest nicotine plasma level ever reported in man [19, 20].

Recently, the oral mucosa permeability of nicotine was measured in vitro, using several regions of porcine oral mucosa [18]. The oral mucosa of the pigs has been known to have a similar morphological structure to that of human oral mucosa in corresponding regions of the oral cavity [21]. Similarity in the permeability of nicotine between porcine and human oral mucosae has been demonstrated [22].

The oral mucosa permeability constant (P_m) can be calculated from the following relationship [1]:

$$P_m = \frac{Q/t}{(C_e - C_c)} \qquad \text{(Eq. 1)}$$

where Q is the cumulative amount of penetrant traversing a unit surface area of the oral mucosa in time t; C_e and C_c are the concentrations of the penetrant on the epithelial and connective tissue sides of the oral mucosa, respectively.

The permeability constant (P_m) obtained for the permeation of nicotine across various oral mucosae, prior to achieving a steady-state, has been found to be time-dependent and increases with time. The results indicated that regions which eventually yield the highest permeability constant of nicotine at steady-state also show a greater rate of increase in permeability constant during the initial phase of permeation across the tissues.

On the other hand, the permeability constant of nicotine across the skin, a keratinized tissue, is low and does not show increase with time. It is interesting to note that the permeability constant of nicotine across gingiva, which is also a keratinized tissue, at steady state is not significantly different from the permeability constant (at 8-hr point) across the non-keratinized buccal mucosa.

It is known that permeability constant is thickness-dependent (Equation 2) and the thickness (h_m) of tissue varies from one region to another; therefore, for better comparison of the permeation characteristics of various tissues the steady-state permeability constants obtained for various tissues should be corrected for the variation in thickness to obtain the normalized permeability (P_m^n):

$$P_m = \frac{KD_m}{h_m} \qquad \text{(Eq. 2)}$$

$$P_m^n = P_m h_m = KD_m \qquad \text{(Eq. 3)}$$

where K is the partition coefficient for the interfacial partitioning of a penetrant molecule from a vehicle to a tissue and D_m is the diffusion coefficient of the penetrant molecule in the tissue.

The results are outlined in Table 1, which indicate that the normalized permeability varies from one oral region to another; and the oral mucosa permeability of nicotine, both apparent and normalized, is 127-371 fold greater than that for the skin.

TABLE 1

Normalized Permeability of Nicotine

Tissue	Characteristics: Thickness[1] (microns)		Characteristics: Surface Layer	Steady-state Permeability[1]: Apparent[2] (cm/sec) x 10^7	Steady-state Permeability[1]: Normalized[3] (cm^2/sec) x 10^{10}
Oral Mucosa					
Buccal mucosa	772	(20)	Non-keratinized	5.62 (0.80)	433.86 (61.76)
Floor of mouth	192	(7)	Non-keratinized	11.63 (0.65)	223.30 (12.48)
Gingiva mucosa	208	(9)	Keratinized	7.13 (0.72)	148.30 (14.98)
Skin					
Abdomen (glabrous)	69	(4)	Keratinized	0.17 (0.02)	1.17 (0.14)

(1) Mean (±SEM)

(2) Calculated from Squier's data (1986) which were generated from pigs by *in vitro* studies.

(3) Calculated from:
Normalized permeability = apparent permeability x thickness

Smoking Cessation by Nicotine Replacement Therapy

Nicotine replacement therapy, which is a therapy involving the systemic administration of nicotine in a non-cigarette form to partially replace the nicotine that would otherwise have been obtained from cigarette smoking, has been developed to minimize the withdrawal syndrome and as aid to smoking cessation. The amount of nicotine administered is approximately 50% lower than that obtained from the smoking of cigarettes, but high enough to reduce withdrawal symptoms. Once smoking abstinence has been achieved, administration of nicotine can be discontinued.

Although nicotine may be associated with some smoking-related illnesses [8], replacement therapy with nicotine is likely to provide a major

health benefit as a result of avoiding the inhalation of more dangerous by-products of tobacco smoking. These by-products, such as tar, have been thought to be associated with smoking-related diseases and have adverse effects on the cardiovascular and pulmonary systems. Furthermore, it is also cost-effective because it is less expensive to prescribe nicotine replacement therapy than to treat the diseases caused by tobacco smoking [23].

Nicotine Replacement Therapy by Oral Nicotine

Nicotine replacement therapy was pioneered with the development of Nicorette® (Marion Merrel Dow), an oral nicotine-releasing polacrilex chewing gum. Each piece of Nicorette® contains 2 mg of nicotine bound to an ion-exchange resin formulated in a buffered chewing gum base.

Following the chewing of Nicorette®, 22.7 (±9.4)% of nicotine dose have been reportedly absorbed through the oral mucosa and 26.0 (±13.8)% swallowed with the saliva. The fraction of nicotine swallowed is subject to hepatic "first-pass" metabolism and provides 7.6 (±3.1)% of nicotine to the systemic circulation, which yields a total bioavailability of only 30.6 (±11.5)% [24]. Hourly chewing of one Nicorette® has achieved a plasma level approximately one-half of that resulted from smoking one cigarette per hour, but without the pronounced peak levels observed immediately following each cigarette smoking (compare Figure 4 with Figure 3). However, it should be pointed out that the systemic nicotine level depends on the vigor, rapidity and duration of chewing.

The nicotine dose swallowed is metabolized mainly by the liver with cotinine and nicotine-1'-N-oxide as the principal metabolites. The metabolism of nicotine following absorption through oral mucosa is qualitatively similar to that from cigarette smoking [25].

It has been marketed since 1984 as an aid to smoking cessation. Although the Nicorette® has been shown to increase abstinence rates in smokers in clinical settings, however, there are a number of problems

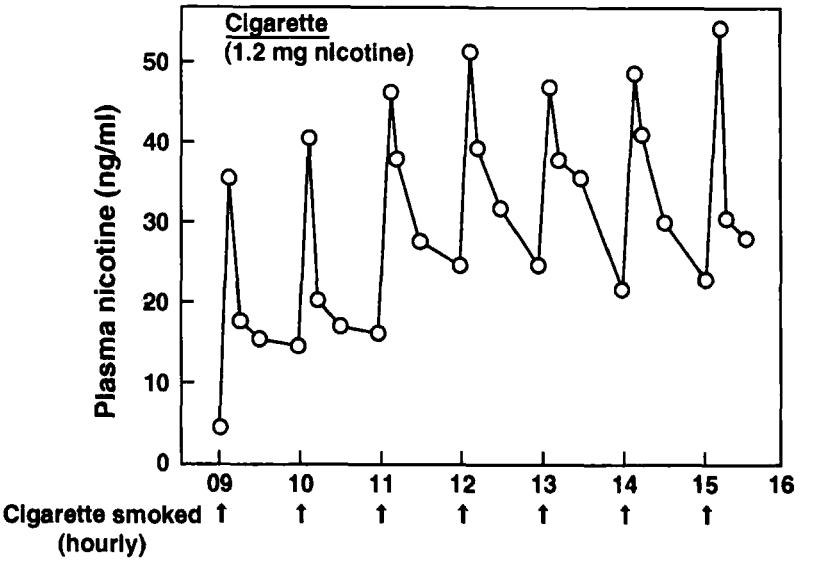

FIGURE 3

The peak and through fluctuation in the plasma profile of nicotine following the hourly smoking of cigarette from 9 a.m. to 3 p.m. [Replotted from the data by Benowitz et al. (1987)].

associated with its use. These include adverse effects (e.g., gastric disturbances), transference of dependence to the gum, inadequate extraction of sufficient nicotine due to improper chewing, absorption being adversely affected by acidic beverages and problems for people wearing dentures [8, 26-28].

Nicorette® is the most thoroughly evaluated form of nicotine replacement therapy [2]. Meta-analysis of 14 randomized, placebo-controlled trials reported that Nicorette® has achieved a significantly higher rate of smoking cessation than placebo gum (27% vs. 18%) after 6-month treatment in the specialized clinics. However, no difference between the medicated and the placebo gums (11.4% vs. 11.7%) has been attained in general medical (community) practices [23, 29]. The most important explanation for this

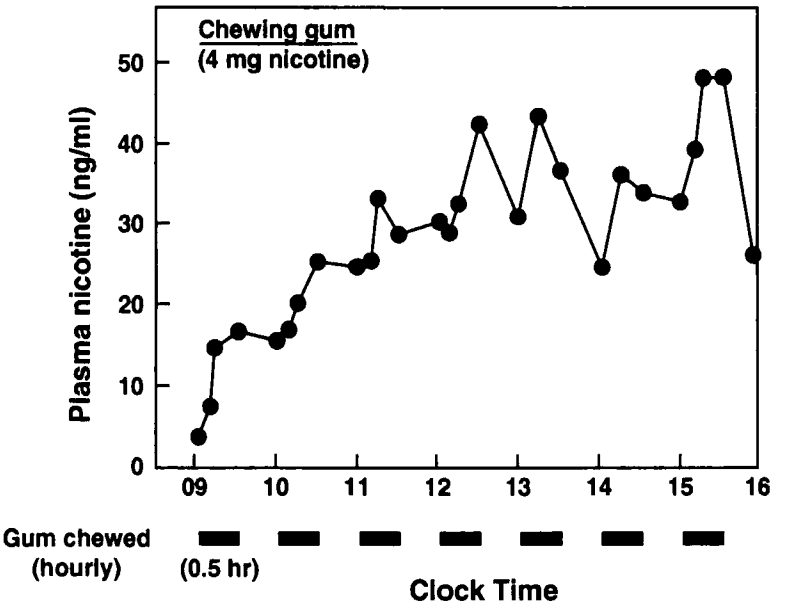

FIGURE 4

The plasma profile of nicotine following the 30-min chewing of Nicorette® gum, one piece per hour, from 9 a.m. to 3 p.m. [Replotted from the data by Benowitz et al. (1987)].

phenomenon may be that subjects treated in clinics are more motivated than those in the community and receive more professional support [29].

Oral administration of nicotine through Nicorette® has the following potential adverse reactions:

a) Local side effects

Mechanical effects of gum chewing may induce traumatic injury to oral mucosa or teeth, jaw ache and eructation.

Changes in oral mucosa result in stomatitis, gingivitis, glossitis and pharyngitis, and aphthous ulcers can occur.

b) Systemic side effects

The reported frequency of adverse effects was highly variable. The only potentially serious adverse effect observed among the 152 patients evaluated

in two clinical studies was cardiac irritability (also a well-known consequence of cigarette smoking).

Since the marketing of Nicorette® in the USA, several deaths and cardiovascular adverse effects have been reported: but, no cause and effect relationship has been established with the use of Nicorette® [25].

If smokers use Nicorette® (2 mg/gum) at a rate of one piece/hr, the cardiovascular effects produced do not differ from those seen with placebo [25].

Therefore, a number of other nicotine delivery systems have been investigated, which include lozenges, nasal sprays and aerosols. Most recently, several transdermal nicotine delivery systems have also been developed for once-a-day nicotine replacement therapy. The efficacy and potential advantages of this method of nicotine replacement therapy as an aid to smoking cessation have been the subject of a recent review [2].

Nicotine Replacement Therapy by Transdermal Nicotine

Transdermal nicotine delivery systems (nicotine-TDDs) deliver nicotine transdermally, which provides a partial replacement of plasma nicotine and reduces the severity of nicotine withdrawal syndrome, and thus allows the smoker to abstain from smoking more easily. The nicotine-TDDs are recommended for daily application for up to 20 weeks, including a series of "weaning-off" courses.

At present, four nicotine-TDDs have received FDA approval for marketing (Table 2). Each system differs in the technology used to achieve the controlled release of nicotine, delivery rate of nicotine, total nicotine loading dose and patch size, as well as the duration of application required. They all consist of an impermeable backing, a nicotine-containing drug reservoir compartment, an adhesive layer, and a peelable protective release liner (Figure 5).

TABLE 2

NDA-approved Nicotine Pharmaceutical Products in U.S.A.

Product	Company/Developer	New Drug Application Submitted	Approved
Transmucosal DDS			
Nicorette®	Merrell Dow/AB Leo	3/17/81	1/13/84
Transdermal DDS			
Nicoderm®	Marion Merrell Dow/Alza	-	11/07/91
Habitrol®	Ciba-Geigy/Lohmann	11/20/90	11/27/91
ProStep®	Lederle/Elan	6/30/91	1/28/92
Nicotrol®	Warner-Lambert/Kabi	1/04/91	4/22/92

Nicotine-TDDs offer an efficient system for the noninvasive systemic delivery of nicotine and have several pharmacokinetic advantages over the traditional routes of nicotine administration:

a) Nicotine is known to be extensively metabolized by the liver, with a systemic bioavailability of only 30.6 (±11.5) % by oral administration. On the other hand, Nicotine-TDDs deliver nicotine directly into the systemic circulation, through the intact skin, and thus eliminate the problem of hepatic "first-pass" metabolism.

b) Nicotine-TDDs achieve a controlled delivery of nicotine, so a steady-state plasma nicotine concentration profile is achieved and maintained (Figure 6), without the intensive fluctuation of peaks and troughs seen with the smoking of cigarette (Figure 3) and the chewing of nicorette® gum (Figure 4).

c) Nicotine-TDDs achieve a plasma nicotine level of approximately half those achieved by smoking (compare Figure 4 with Figure 3). This

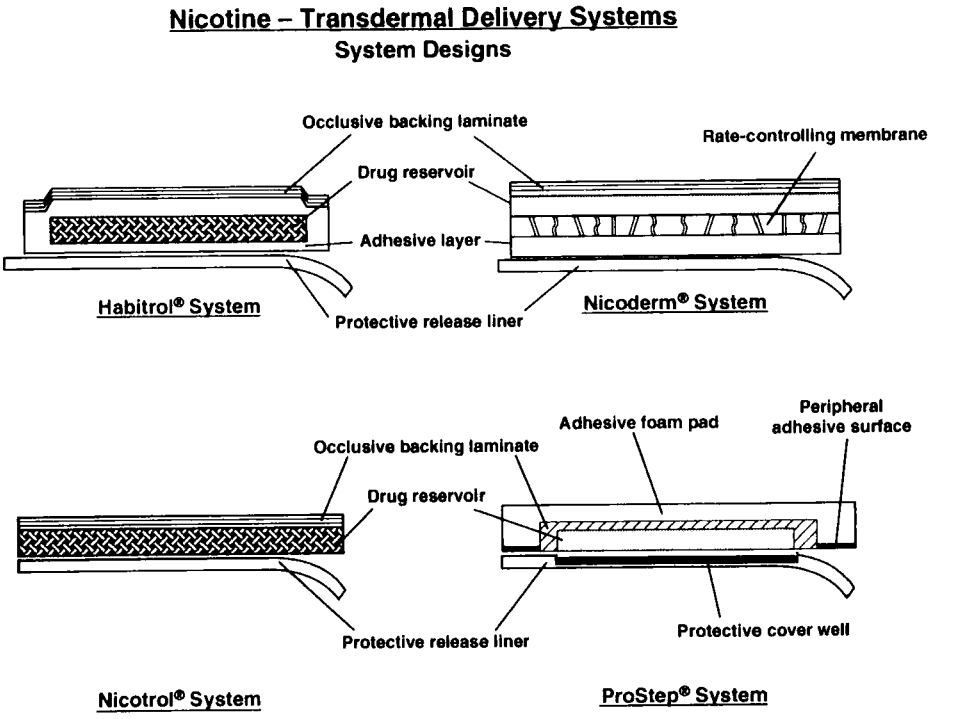

FIGURE 5

The cross-sectional view of the four NDA-approved nicotine-releasing transdermal delivery systems (nicotine-TDDs) recently marketed in the USA.

partial replacement of plasma nicotine is capable of alleviating a number of the nicotine withdrawal symptoms associated with the cessation of smoking and enables the smokers to abstain from smoking more easily. Furthermore, due to their pharmacokinetic profile, they probably have a low dependency potential.

Nicotine-TDDs were selected as the product of the year in 1992 (Fortune, 12/28/92) with Habitrol® as the best-seller, which has taken over 46% of the $800 million market.

Pharmacodynamic Activities

Nicotine binds to cholinergic nicotinic receptors in the central and peripheral nervous systems. Its actions in the central nervous system (CNS) are thought to be important in the development of nicotine addiction. Nicotine has multiple effects in the brain and activates several neurochemical pathways, the most relevant one probably being the enhancement of mesolimbic dopaminergic function. Additional CNS actions lead to increase in arousal, attention and reaction time, as well as decrease in anxiety and stress reactions.

Peripheral effects observed include increase in some cardiac variables, like heart rate, blood pressure, cardiac output and coronary blood flow, changes in endocrine and metabolic functions, cutaneous and systemic vasoconstriction, as well as a decrease in muscular tone. Nicotine also appears to be involved in the regulation of body weight, possibly by increasing metabolism and energy consumption.

The pharmacodynamic effects of nicotine delivered transdermally from the nicotine-TDDS appear to be similar to, or less pronounced than those observed following smoking [2].

Clinical Pharmacokinetic Profiles

Nicotine-TDDs are designed to control the systemic delivery of nicotine at a relatively constant rate and thus achieve a more steady plasma concentration of nicotine during the period of its application, even though troughs occur at the end of a dosing period which appear to remain still above the nicotine levels required for the alleviation of withdrawal symptoms (Figure 6). Application of the nicotine-TDDs with varying dosage rate has resulted in an increase in plasma nicotine concentrations proportional to the dosage rate, following the observation of a time lag of 0.25 - 4.0 hrs [2]. Steady-state plasma nicotine concentrations of 10-23 ng/ml are achieved within 2-4 days of repeated applications (Figure 7), which are approximately half of those

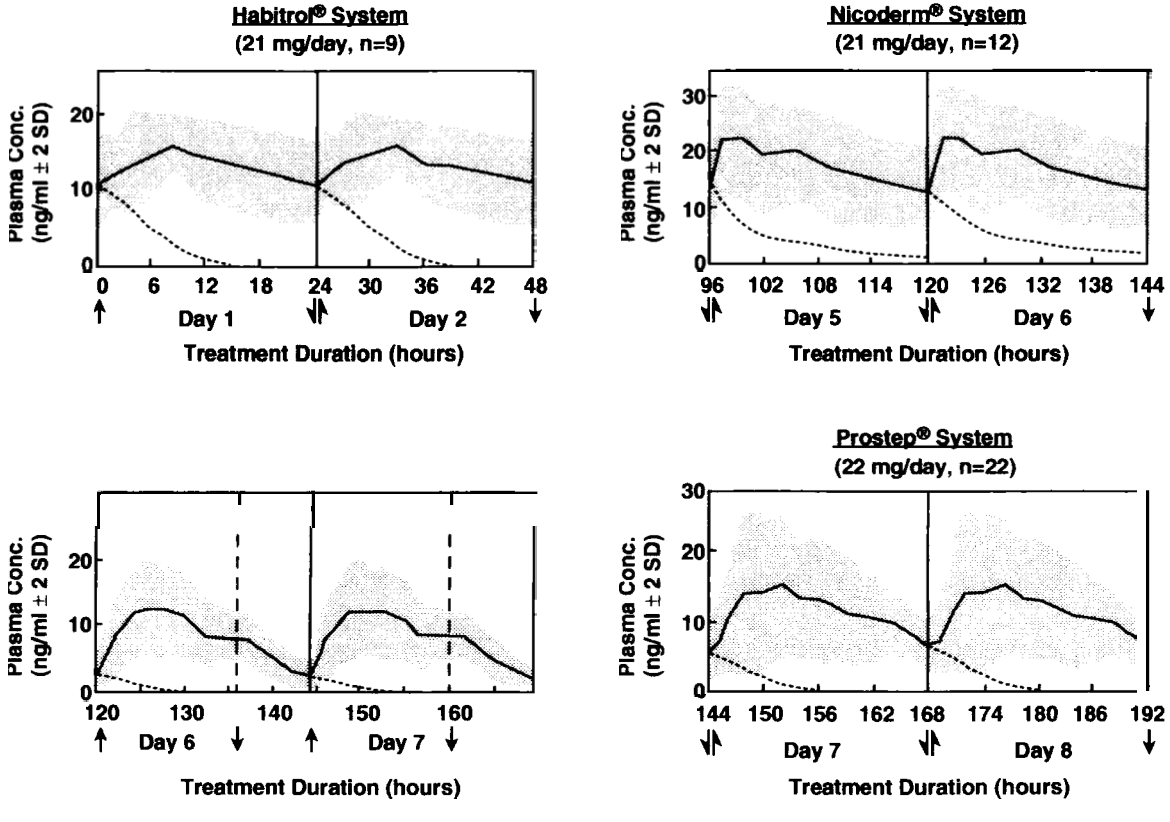

FIGURE 6

Comparison in the plasma profiles of nicotine following the two consecutive daily applications of various marketed nicotine-TDDs

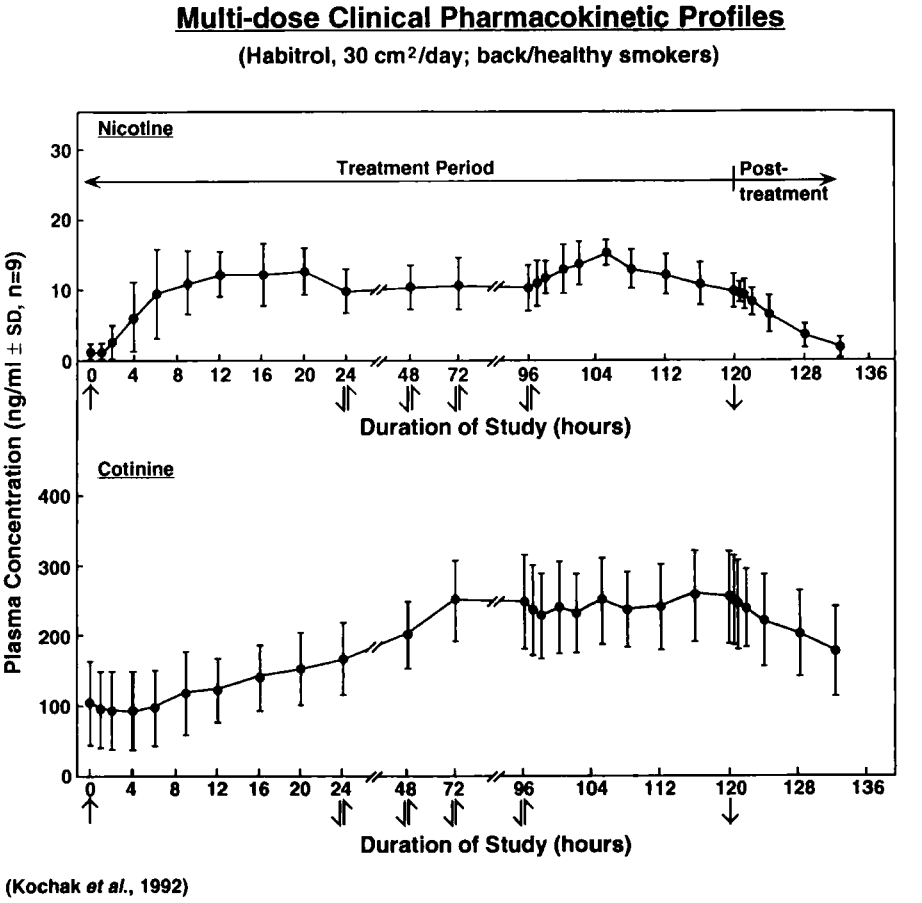

FIGURE 7

The clinical pharmacokinetic profiles of nicotine and its major metabolite, cotinine, in 9 healthy male smokers following the multiple-dose repeated daily application of Habitrol® system (30 cm^2 patch with a nominal rate of 0.7 $mg/cm^2/day$) for 5 days [Replotted from the data by Kochak et al. (1992)].

seen after ad libitum smoking over 15 hours (Figure 3). The site of application does not appear to markedly affect the transdermal absorption of nicotine [2].

Nicotine was found to be extensively metabolized, primarily in the liver, with the formation of cotinine as the major metabolite. Cotinine is

present in the plasma in higher concentrations than nicotine (Figure 7) but does not appear to contribute to the pharmacodynamic effects of nicotine. Following repeated application, approximately 10% of the nicotine administered transdermally is excreted unchanged in the urine, while another 10-15% is eliminated as cotinine and 45% as 3-trans-hydroxycotinine via the urinary excretion. The nicotine delivered transdermally was observed to have an elimination half-life of 3-6 hrs. Attainment of constant AUC values suggests that nicotine does not accumulate in the body; but skin depots may develop at the site of application and result in some continued absorption of nicotine even after the removal of nicotine-TDDs [2].

Since nicotine-TDDs produce a gradual increase in plasma nicotine concentrations, their capability of substituting for the rapid infusion of nicotine attained by cigarette smoking has been questioned. Smokers attempting to quit may find that the application of a nicotine-TDD does not provide the nicotine 'high' that is obtained from a cigarette and, therefore, does not satisfy their craving. On the other hand, the gradual rise in plasma nicotine concentration is also likely to ensure that the smoker does not become dependent on the system.

Therapeutic Efficacy

The efficacy of nicotine-TDDs as an aid to smoking cessation has been evaluated in a number of placebo-controlled trials, and some of these trials also included concomitant behavioral counselling and therapy, in healthy smokers who were motivated to quit smoking. Abstinence rates of 30-41% have been reported during the first 6 weeks of treatment in the subjects without behavioral support, compared with 4-21% in placebo recipients. Higher initial abstinence rates (35-86%) have also been achieved in subjects receiving concomitant supportive counselling or therapy. However, abstinence rates tend to decrease with time, to approximately half the initial rates at 6-12 months after the initial period of treatment [2].

Transdermal nicotine treatment has significantly reduced the daily usage of cigarette even in those subjects who were unable to totally abstain from smoking, and attenuated some nicotine withdrawal symptoms, including cigarette craving, but has little effect on the body weight gain that often occurs following smoking cessation [2].

The ease of use and unobtrusive nature of the nicotine-TDDs have resulted in a high degree of patient compliance. They offer a convenient form of nicotine replacement therapy. Majority of users (>80%) have rated nicotine-TDDs as acceptable, convenient and comfortable to use [30, 31]. The short-term abstinence rates achieved with this therapy are encouraging. However, the maintenance of abstinence in the long-term is harder to achieve; but the transdermal nicotine replacement therapy represents an important advancement in the difficult area of smoking cessation management [2].

Clinical Tolerability

A significant proportion of patients experience dermatological adverse reactions with the nicotine-TDDs. These usually consist of mild to moderate erythema and transient itching at the site of application, which can be easily treated with topical steroids or oral anti-histamines [2]. Severe skin irritation, such as eczema, generalized rashes and sensitization, have occasionally been reported, and have led to the discontinuation of transdermal nicotine therapy in 1.4 - 7.1% of the patients treated [2]. However, the systems have the advantage of being easily removed from the site of application if adverse effects are observed.

Systemic adverse reactions, such as CNS, gastric and sleep disturbances, sweating and cough, etc., have been reported. There appears to be some risk of adverse cardiovascular reactions in patients who continue to smoke during the application of nicotine-TDDS. The nicotine-TDDS's are expected to have a low dependency potential due to their pharmacokinetic profile, such as low plasma nicotine concentration achieved, and removal of the link between the craving and the self-administration of the systems.

The adjustment of concomitant medications may be necessary due to the elimination of the metabolic and pharmacodynamic effects of nicotine following the cessation of smoking.

Dosage and Administration

There appears to be no consensus regarding the optimal period for the daily applications of nicotine-TDDs. Three of the currently available nicotine-TDDs (Nicoderm®, Habitrol® and Prostep®) have been designed for 24-hr application, while the remaining system (Nicotrol®) is prescribed for 16-hr application (during the waking hours only) (Figures 5 & 6). The 16-hr system has been designed with the intention of reducing the occurrence of undesirable effects, such as sleep disturbance, and the possibility of tolerance development. However, early morning relapse may be a problem with the 16-hr regimen [33]. In any case, both regimens appear to achieve equivalent abstinence rates.

The initial transdermal nicotine dosage recommended for assisting the cessation of smoking is up to 22 mg/24 hr or 15 mg/16 hr, even though lower dosages are recommended for lighter smokers, those with cardiovascular disease, and for those who weigh less than 45 kg. Some dose adjustment may also be required in the first 2 weeks of the treatment. The nicotine-TDDs should be applied daily for 4-12 weeks, followed by a "weaning" course of 2-8 weeks, during which the dose may be reduced stepwise to 7 mg/24 hr or 5 mg/16 hr, and eventually discontinued. With one of the systems, the weaning period is optional and therapy may be discontinued when smoking cessation has been achieved [2].

Comparative Evaluation of Transdermal Nicotine Delivery

Since the technical data supplied in the product information and patient instructions for the four marketed nicotine-TDDs were collected under different study conditions, a question has been raised on the bioequivalence

and interchangeability of these TDDs, especially when a nicotine-TDD originally prescribed has run out of its supply. No scientific data are available for medical or pharmaceutical profession to make a sound judgment for product substitution. As conducted previously in this laboratory for various nitroglycerin-TDDs [34], a systematic series of kinetics studies were recently initiated again to investigate and compare the mechanisms and rate profiles of the release kinetics of nicotine from these TDDs and its subsequent transdermal delivery through the skin under identical conditions [35].

1) Release kinetics of nicotine

In order to gain some insights into the mechanisms involved in the release of nicotine from the four marketed nicotine-TDDs and their roles on the transdermal delivery of nicotine, the drug release kinetics studies were conducted, for which nicotine in the various TDDs was released into a solution medium with pH simulating the skin surface pH (5.0). The results indicated that while Habitrol®, Nicoderm® and Nicotrol® systems release the nicotine loading gradually, at a nonlinear but controlled manner, throughout the course of release studies, Pro-step® system releases all its nicotine loading rapidly within only one hour. The release profiles shown in Figure 8 suggests that except Nicoderm® system, which appears to release nicotine at Q vs. t kinetics [resulted from the polymer membrane permeation-controlled process (Figure 5)] during the steady-state period (2-12 hrs), the release profiles of nicotine from other systems all follow the polymer matrix diffusion-controlled process as shown by the biphasic linear Q vs. $t^{1/2}$ relationship. These relationships are presented mathematically as follows [1]:

$$Q = \frac{K_{p/r} K_{a/p} D_a D_p}{K_{p/r} D_p h_a + K_{a/p} D_a h_p} C_r t \qquad \text{(Eq. 4)}$$

where Q is the cumulative amount of nicotine released from a unit area of the nicotine-releasing surface of each TDD; C_r is nicotine concentration in the

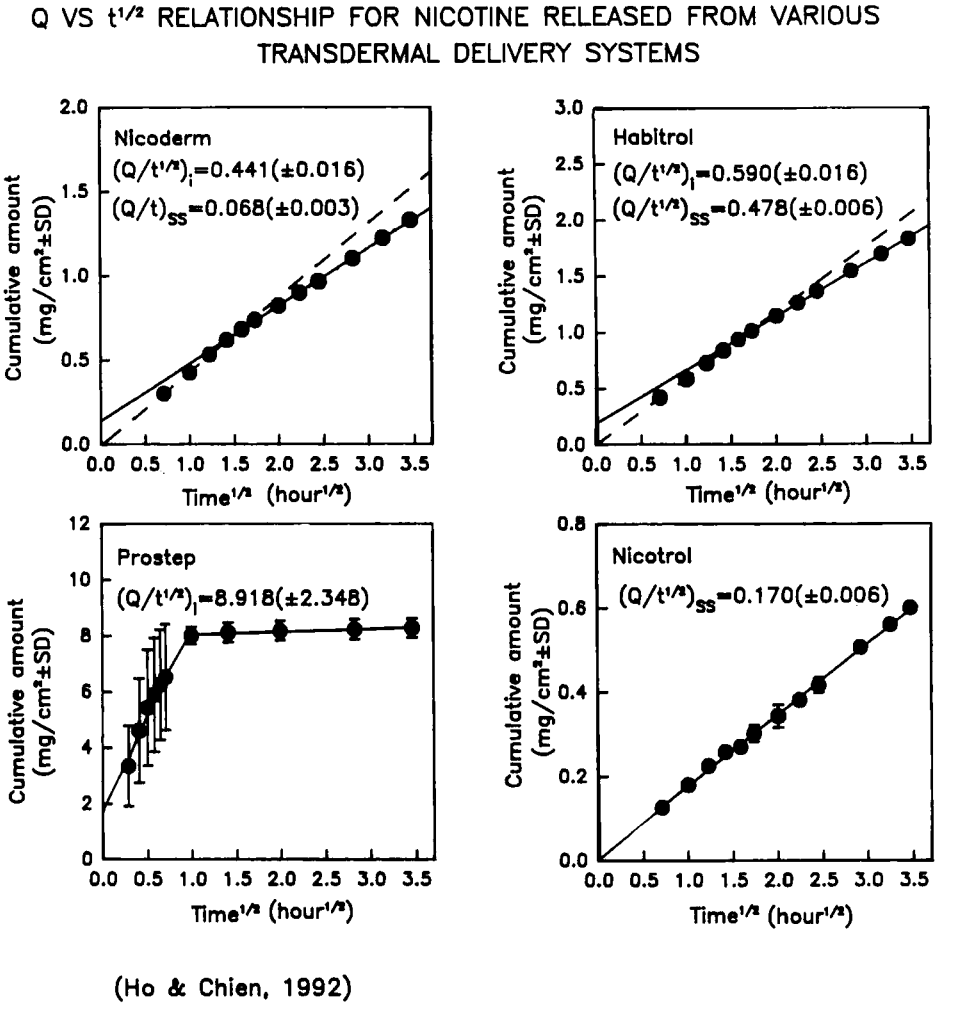

FIGURE 8

Comparison in the Q vs. $t^{1/2}$ release profiles of nicotine delivered from the four recently-marketed nicotine-TDDs.

drug reservoir compartment; $K_{p/r}$ and $K_{a/p}$ are the partition coefficients for the interfacial partitioning of nicotine from the reservoir compartment to the rate-controlling polymer membrane and from the polymer membrane to the surface adhesive layer, respectively; D_p and D_a are the diffusion coefficients in the polymer membrane and in the adhesive layer with the respective thickness of h_p and h_a, respectively.

$$Q = [(2L_d - C_p) C_p D_p]^{1/2} t^{1/2} \qquad \text{(Eq. 5)}$$

where L_d is nicotine loading dose in a unit volume of the drug reservoir compartment; C_p and D_p are the solubility and diffusivity of nicotine in the polymer matrix, respectively.

The unique release profiles observed in Figure 8 is apparently a contribution of the differences in system design and structural composition of these nicotine-TDDs (Figure 5). It is known that Nicoderm® system is constructed from a nicotine reservoir, which is dispersed in a (ethylene/vinyl acetate) copolymer matrix, covered by a rate-controlling polyethylene membrane having a polyisobutylene adhesive surface. Nicotine is also purposely dispersed in the surface adhesive layer to achieve a rapid initial release. Thus, this system releases nicotine initially in $\underline{Q \text{ vs. } t^{1/2}}$ manner and then shifts to $\underline{Q \text{ vs. } t}$ pattern. Prostep® system, which has nicotine dispersed in a hydrogel matrix surrounded by a peripheral adhesive pad, releases the nicotine dose at a very high release flux when the system comes into contact with the solution medium. Habitrol® system has nicotine dissolved in a methacrylic acid copolymer solution and dispersed in a pad of non-woven viscose and cotton, on which adhesive layer is laminated as the drug-releasing surface. Nicotrol® system has nicotine dissolved in a rate-controlling adhesive polymer dispersing in a structural non-woven material. Although Habitrol® and Nicotrol® systems share some similarities in system design, the variations in structural composition gave Habitrol® system a biphasic release pattern, with initial release flux of 0.590 (±0.016) and a steady-state flux of 0.478

(±0.006) mg/cm^2/hr$^{1/2}$, while Nicotrol® system produced a monophasic release pattern, with a substantially lower steady-state flux of only 0.170 (±0.006) mg/cm^2/hr$^{1/2}$ (Figure 8). The biphasic release profile of nicotine from Habitrol® system could be attributed to the possibility that nicotine diffusing into the surface adhesive layer during storage is released at first, which is then followed by a matrix diffusion-controlled release of nicotine from the copolymer matrix in the reservoir compartment.

2) Skin permeation kinetics of nicotine

The skin permeation kinetics of nicotine delivered from these marketed nicotine-TDDs was investigated using both human cadaver and hairless rat skins. The results in Figure 9 indicate that a biphasic Q vs. t permeation profile has been observed for the human cadaver skin permeation of nicotine delivered from all four TDDs. The transdermal delivery of nicotine from these nicotine-TDDs is always faster in the beginning (with a rate in the range of 0.068-0.289 mg/cm^2/hr), which lasts for 6-16 hours, and then shifts to a lower rate of permeation, which ranges from 0.029 to 0.165 mg/cm^2/hr, for the remaining period of the 30-hr study. It is interesting to note that the steady-state permeation rates obtained from Habitrol® and Nicoderm® systems (0.071 and 0.072 mg/cm^2/hr) are in good agreement with those for hairless rat skin (0.073 mg/cm^2/hr).

3) Correlation of skin permeation with release kinetics

The observation of constant skin permeation profiles for nicotine released from Habitrol®, Nicotrol® and Pro-step® by Q vs. $t^{1/2}$ kinetics is apparently due to the fact that the stratum corneum is a non-sink medium and thus serves as a rate-limiting barrier for the transdermal delivery of nicotine released to the stratum corneum surface from the nicotine-TDDs.

On the other hand, the skin permeation rate of nicotine delivered from Nicoderm® system, which is a polymer membrane permeation-controlled drug

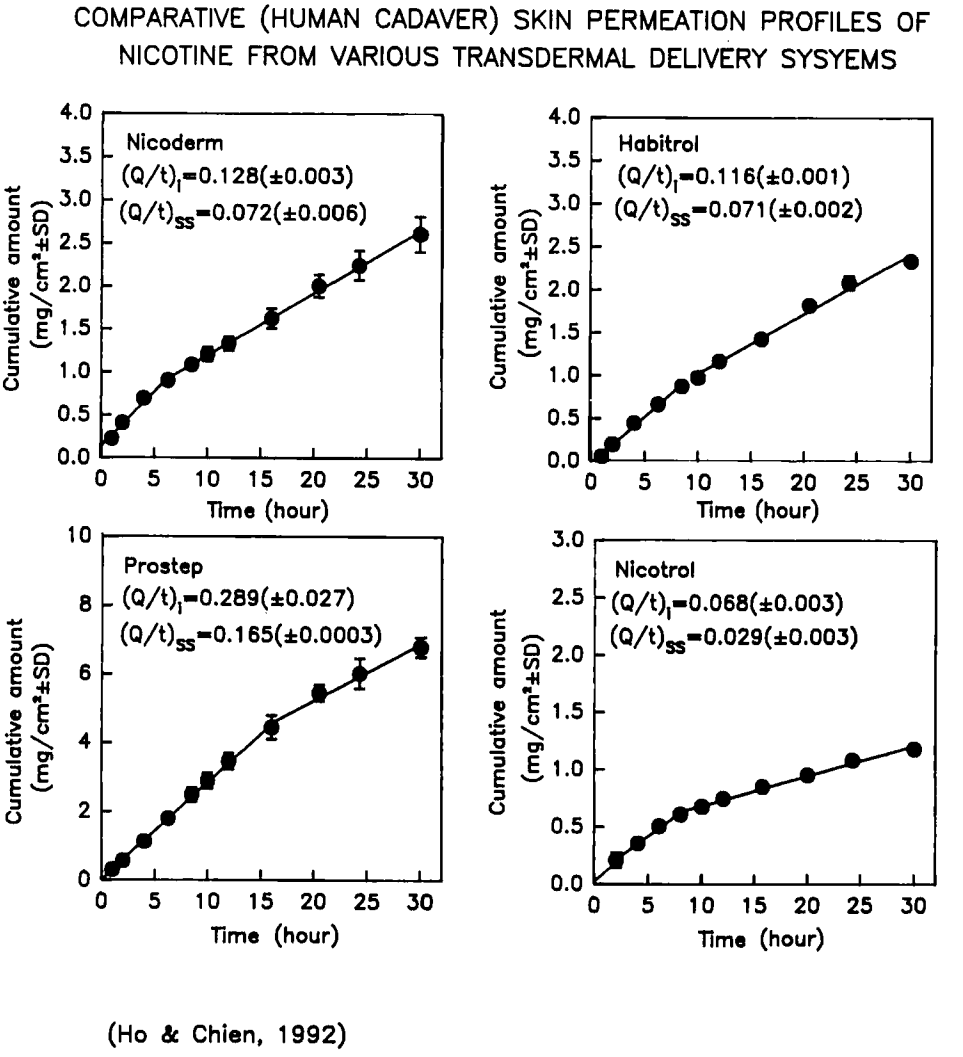

FIGURE 9

Comparison in the Q vs. t permeation profiles of nicotine through human cadaver skin following the controlled release from the nicotine TDDs.

delivery system, was found to be very close to the rate of release [0.072 (±0.006)] vs. 0.068 (±0.003) mg/cm^2/hr]. The agreement achieved suggests that at steady state, the skin permeation kinetics of nicotine from this system is primarily controlled by the release of nicotine from the system.

In conclusion, the in vitro skin permeation data generated, using human cadaver skin, suggests that Habitrol® and Nicoderm® systems could be considered bioequivalent and are thus interchangeable; but, Pro-step® and Nicotrol® systems are neither bioequivalent to Habitrol® nor Nicoderm® system and thus none of them are interchangeable to one another.

New Developments of Interest

As reported above, the 4 marketed nicotine-TDDs have shown to provide effective assistance to smokers undergoing smoking cessation program with minimal occurrence of withdrawal symptoms. Unfortunately, substantial skin reactions have been reported with these nicotine-TDDs [2]. The most common skin irritation is a mild short-lived erythema, pruritus or burning at the application site, which was seen at least once in 35, 47, 47 and 54 of the subjects on Habitrol®, Nicoderm®, Nicotrol® and Prostep® systems, respectively, in clinical trials [36-39]. After removal of these systems, local erythema was observed to occur at least once in 17, 14, 7 and 22% of the users, respectively, and local edema, at lower frequencies, was also observed [36-39].

To reduce the degree of skin irritations, a new type of nicotine-TDD has been recently developed by TBS Laboratories, Inc. (Piscataway, New Jersey, USA). It is a multi-laminate adhesive-type transdermal nicotine delivery system having nicotine and counterirritant dispersed as microreservoirs, in the adhesive polymer [40]. In vitro skin permeation kinetics studies demonstrated that this nicotine-TDD yields a constant skin permeation profile (Figure 10) with a permeation rate of 0.068 (±0.003) mg/cm^2/hr across human cadaver skin and 0.059 (±0.014) mg/cm^2/hr across

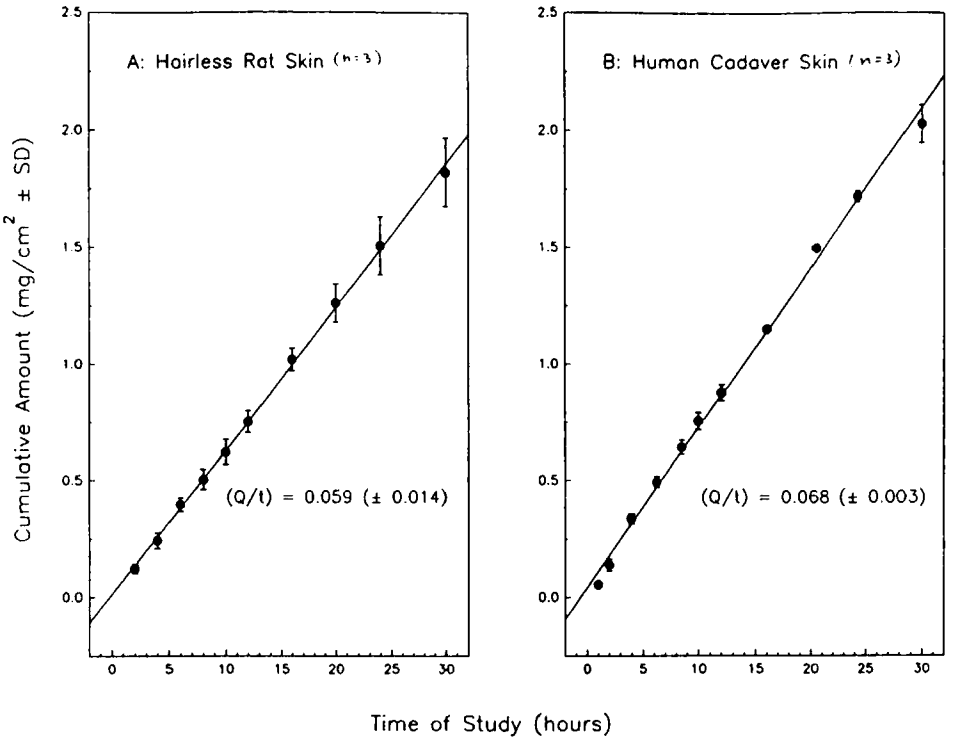

FIGURE 10

Comparative in vitro skin permeation profiles of nicotine, delivered from the TBS' nicotine-TDD, through hairless rat skin (A) and human cadaver skin (B).

hairless rat skin, which are comparable to the steady-state permeation rates attained by Habitrol® and Nicoderm® systems [41].

It was evaluated clinically with 25 subjects who completed a 4-wk study protocol [42]. The results indicated that skin reactions are generally very mild, with 88% of the subjects having an average reported itchiness rating of less than or equal to 1.0 ("mild") and no subject reported average rating greater than 1.66 (using a 4-point scale). Average ratings of redness at the site of patch application are less than or equal to 1.0 ("mild") in 76% of the

subjects and no subject reported average rating greater than 1.16. Furthermore, 10 of the 25 subjects (40%) reported complete abstinence from smoking for the entire duration of the 4-wk trial [42].

Two phase I clinical studies were carried out recently, using the same FDA-approved study protocol, in two ethnic groups: 32 American smokers and 33 Taiwanese smokers, respectively, at the Nicotine Research Laboratory, Duke University Medical Center (Durham, North Carolina) [42] and at the Department of Psychiatry, National Taiwan University Hospital (Taipei, Taiwan) [43]. The results indicated that steady-state nicotine plasma levels have been achieved in both groups of smokers within 4-6 hrs and maintained throughout the course of 24-hr topical application of this newly-developed nicotine-TDD (Figure 11), while the plasma concentrations of nicotine were observed to attain the peak levels after taking the last dose of Nicorette® gums [each of 6 doses (2 mg each) was taken hourly and chewed for 30 min] and then decline rapidly by first-order elimination kinetics. Furthermore, the steady-state plasma concentrations of nicotine achieved by the transdermal controlled delivery of nicotine from this nicotine-TDD (20 cm^2) in both ethnic groups are very similar [i.e., 8.33 (±0.74) ng/ml (for Americans) and 8.35 (±0.67) ng/ml (for Taiwanese)]. The peak plasma levels of nicotine attained by oral chewing of Nicorette® gums in Taiwanese smokers are higher than that in American smokers [11.76 (±1.31) vs. 7.37 (±1.22) ng/ml].

The relationship between the plasma profiles of nicotine delivered transdermally and the drug-releasing area of TBS' nicotine-TDD is displayed in Figure 12, which indicates that following the topical application of nicotine-TDD, plasma concentrations of nicotine show a rapid exponential increase, during the period of transdermal absorption phase, for all 3 dosage levels and a dosage-dependent rate of increment in nicotine concentrations is observed. Steady-state nicotine levels are attained within 4-6 hours for all 3 nicotine-TDDs, with respective drug-releasing surface of 10, 20 and 30 cm^2, in both American and Taiwanese groups of smokers. There appears no statistically-

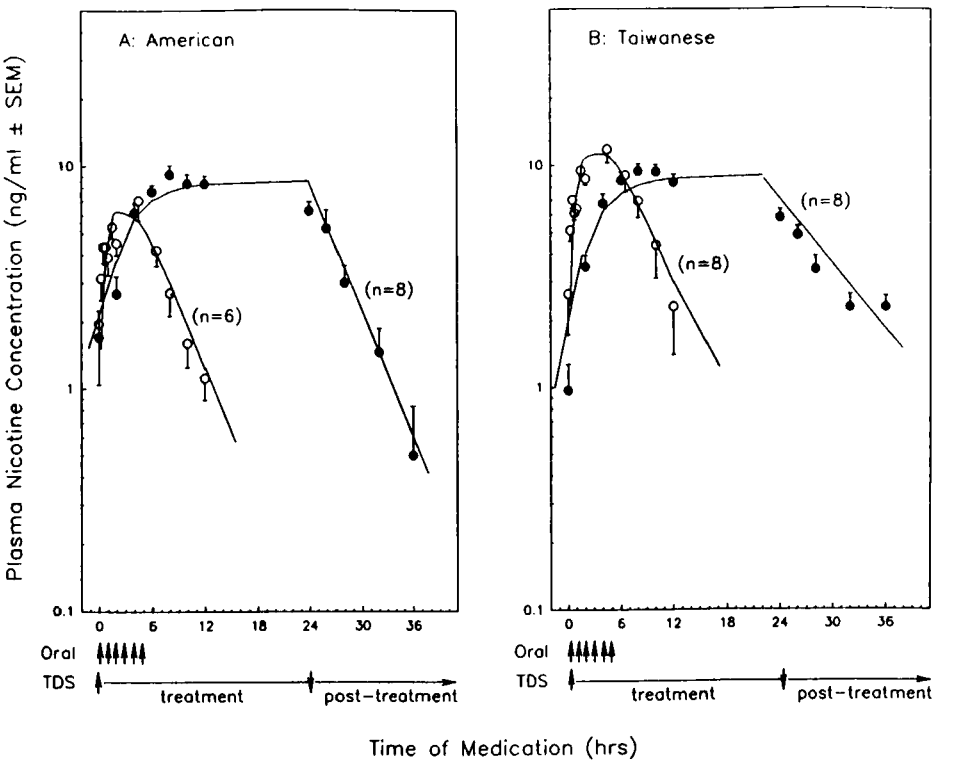

FIGURE 11

Comparative plasma profiles of nicotine following the single-dose topical application of nicotine-TDD (20-cm^2) and multiple-dose oral chewing gum in American (A) and Taiwanese (B) smokers. Keys: (○) oral (Nicorette®, 6 x 2 mg/hr) and (●) transdermal (TBS, 1 x 20-cm^2/24 hrs).

significant difference in the dose proportionality of steady-state plasma levels achieved between these two ethnic groups.

The residual amounts of nicotine in the nicotine-TDDs were assayed, following the 24-hr application, and the daily doses of nicotine delivered to the smokers from each unit of nicotine-TDD was determined. The results in Figure 13 show that a linear relationship is established between the daily dosage of nicotine delivered and the drug-releasing area of nicotine-TDD in

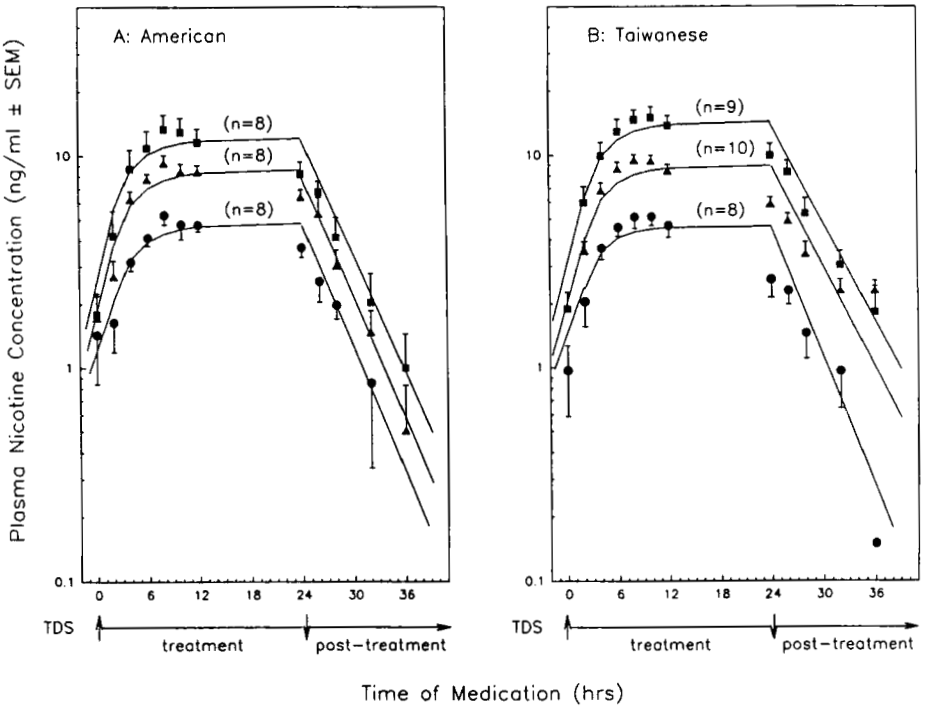

FIGURE 12

Transdermal plasma profiles of nicotine as a function of the patch size of TBS' nicotine-TDD in American (A) and Taiwanese (B) smokers. Keys: (●) 10-cm^2, (▲) 20-cm^2, and (■) 30-cm^2.

both ethnic groups, but a statistically-significant demographic effect is observed in the daily dosage of nicotine delivered to the smokers ($p < 0.05$). It appears that the nicotine doses delivered to Taiwanese smokers from all patch sizes of nicotine-TDDs are considerably greater than those delivered to American smokers (with a ratio of 1.22). The calculated in vivo delivery rate of nicotine to Taiwanese smokers [0.041 (±0.002) mg/cm^2/hr is also significantly greater, statistically, than that of American smokers [0.033 (±0.001) mg/cm^2/hr] ($p < 0.05$).

To evaluate the bioequivalence between these two ethnic groups, the area under plasma nicotine concentration curves (AUC's) in the period of 0-

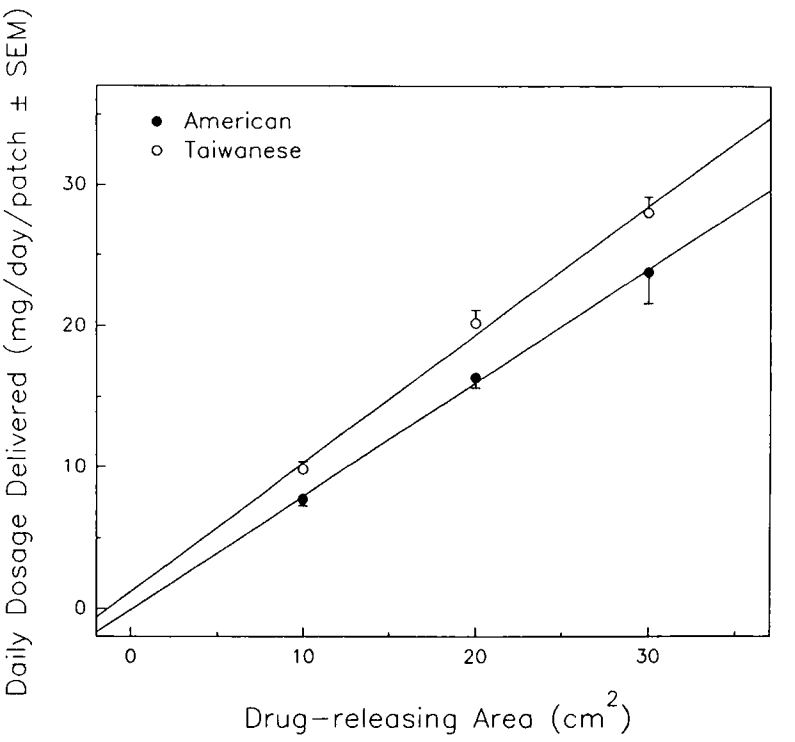

FIGURE 13

Linear relationship between the daily dose of nicotine delivered transdermally from the 24-hr application of TBS' nicotine-TDD, in American and Taiwanese smokers, and the drug-releasing area of nicotine-TDD.

36 hrs were computed, using trapezoidal rule. A linear relationship between AUC's and daily dosages of nicotine delivered transdermally is obtained for both groups (Figure 14), but no significant difference is observed between American and Taiwanese smokers.

In view of the difference in body weight between American and Taiwanese subjects [72.4 (±18.1) vs. 65.6 (±10.3) kg], which could contribute to the variation in steady-state plasma level (C_p) and AUC values, the daily nicotine dose delivered to each subject was normalized by his/her body weight

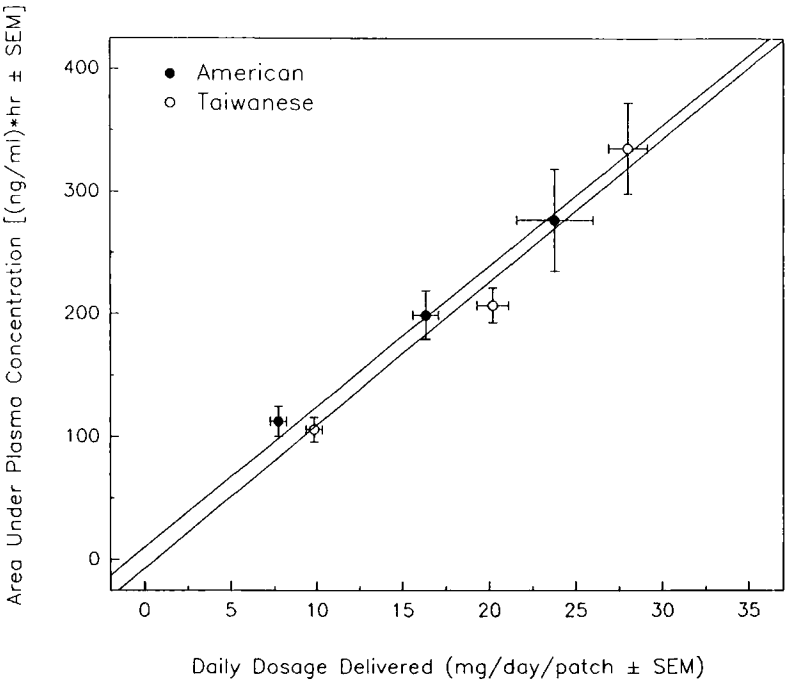

FIGURE 14

Transdermal bioavailability of nicotine, expressed as the area under plasma concentration (AUC), from the 24-hr application of TBS' nicotine-TDD in both American and Taiwanese smokers as a function of the daily dosage delivered from the nicotine-TDD.

to calculate the value of daily dose per kg of body weight. The results suggest that both the C_p and AUC values are proportionally increased with the increase in the normalized daily nicotine dose (Figure 15). However, no significant demographic difference can be assessed. Thus, the difference observed in the in vivo delivery rates of nicotine between American and Taiwanese smokers could be attributed to the difference in the biophysicochemical characteristics of the skin between the two ethnic groups.

It should be pointed out that phase II clinical studies have also been completed in both ethnic groups, using the same FDA-approved study

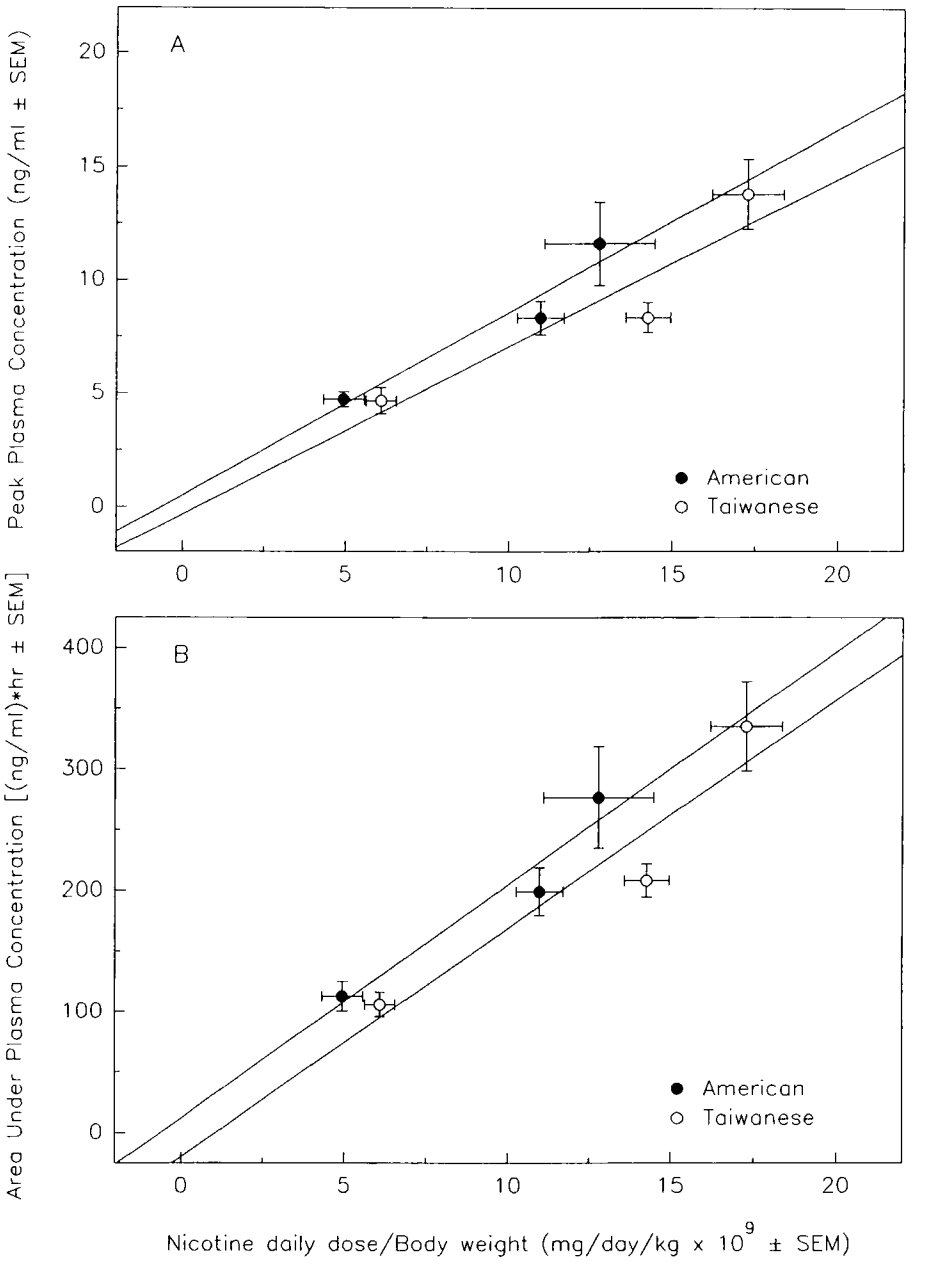

FIGURE 15

Demographic effect on the linear relationship between the daily nicotine dose delivered per unit of body weight and peak plasma nicotine concentrations (A) as well as the area under plasma nicotine concentration (B) in both American and Taiwanese smokers.

protocol, in the same institutions. Furthermore, a bioequivalence study has recently been completed in 18 non-smoking Taiwanese to compare the systemic bioavailability and pharmacokinetic profile of nicotine delivered transdermally from the nicotine-TDDs manufactured by the technology licensee (Sintong Chemical Industrial Co., Ltd./Taoyuan Taiwan) in comparison with that from the technology developer (TBS Laboratories, Inc./Piscataway, New Jersey, USA), using the marketed Habitrol® system as the reference product [44]. Some of the results will be reported at this symposium by Professor R. R-L. Chen.

NONINVASIVE SYSTEMIC DELIVERY OF BIOPHARMACEUTICALS

"Biopharmaceuticals", a term being used to classify those pharmaceuticals produced biologically and/or by biotechnological processes, which are different from the organic-based pharmaceuticals manufactured by chemical synthesis. In this article, the term "Biopharmaceuticals" is used specifically to define the peptide- and protein-based pharmaceuticals. The systemic delivery of these peptide-/protein-type biopharmaceuticals presents additional technical issues which are very different from that for the systemic delivery of organic-based pharmaceuticals, such as nicotine, which has been discussed in the sections outlined above. The systemic bioavailability of peptide-/protein-type biopharmaceuticals is extremely low, when taken orally, and they need to be administered by parenteral routes to achieve their therapeutic activities.

A major challenge encountered in the systemic delivery of peptide-/protein-type biopharmaceuticals is the need to overcome the enzymatic barrier that limits the availability of biopharmaceutical molecules to their target tissues. Degradation usually begins at the site of administration and can be extensive. Even by parenteral (IM or SC) administration, less than complete bioavailability can be achieved. For instance, the SC injection of thyrotropin-releasing hormone, a simple and small tripeptide molecule, in

mice has achieved a systemic bioavailability of only 31.1% [45]. On the other hand, the SC administration of leuprorelin, a stable synthetic analog of LHRH, and of insulin, a structurally more complex protein molecule, has attained a bioavailability of 65 and 80%, respectively [46, 47]. Furthermore, proinsulin, which is converted to insulin in the beta cell by the action of two proteases during the maturation of secretory vesicles [48, 49], was found to degrade extensively in subcutaneous tissues to four metabolites and none of them was insulin [50].

Peptide- and protein-type biopharmaceuticals are subjected to degradation by numerous enzymes (or enzyme systems) throughout the body. This degradation can occur in either one of the following two ways: (i) proteolysis, which is the hydrolytic cleavage of peptide bonds by proteases, such as enkephalinases, and (ii) biochemical modification of the peptide (or protein) molecule, which causes the molecule to denature by aggregation or fragmentation, such as oxidation by glucose oxidase and phosphorylation by kinases. However, proteolysis is by far the more common one. There is usually a rate-limiting event in each degradation process. Beyond this initial event, peptide or protein molecules are rapidly degraded to small peptides and then further metabolized to amino acids.

The enzymatic barrier for the transmucosal permeation of peptide-/protein-type biopharmaceuticals has been investigated. For example, enkephalins are biologically-occurring pentapeptides that act as neurotransmitters or neuromodulators in pain transmission [51, 52] and thus have analgesic properties. However, their analgesic activity is rather short in duration, with a half-life of less than 1 min, and remains purely transient even by intracerebroventricular administration. This rapid loss in activity has reportedly resulted from their rapid inactivation by enzymes present in plasma [53, 54], brain membrane and brian homogenate [55, 56], calf and bovine brain [57, 58], rat and mouse brain extracts [59], and other tissues [60-62]. The results accumulated to date have suggested that several enzymes are

responsible for the degradation of enkephalins, and their relative contribution to the total amount of enkephalins metabolized varies from one enzyme to another. Differences also exist from one species to another and even among various tissues or preparations within a given species.

The hydrolysis of enkephalins was studied in the homogenates of various rabbit mucosal tissues, and the results indicated that enkephalins are most rapidly hydrolyzed in the homogenates from buccal and rectal mucosae, followed by nasal and then vaginal homogenates, but the difference in their hydrolysis rates is small [63]. Aminopeptidases were found to be the major enzymes responsible for the hydrolysis of both leucine- and methionine-enkephalins, while dipeptidylpeptidase and dipeptidylcarboxypeptidase contributed to a much lesser extent. In the homogenate of anterior segment tissues from the albino rabbit's eye, these enkephalins were found to be equally susceptible to hydrolysis. Peptidases were also involved in their hydrolysis in these ocular tissues in a similar manner as in mucosal homogenates [64]. These results led to the conclusion that a similarity exists in the magnitude of enzyme activities among various mucosal routes, in terms of the rate constants for the hydrolysis of methionine-enkephalin in the homogenates of various mucosal tissues [65]. In contrast, dipeptidylcarboxypeptidase was found to be the primary enzyme responsible for the hydrolysis of [D-ala]^2-Met-enkephalinamide, a synthetic analog of methionine-enkephalin that was designed to be more resistant to the hydrolysis by aminopeptidase, in the homogenates of these non-oral mucosae [63, 64]. It has also been reported that in the rat, endopeptidase activity toward [D-ala]^2-Met-enkephalinamide is significantly lower in buccal than in intestinal homogenates; in the hamster, on the other hand, there is no significant difference in activities between buccal and intestinal homogenates [66].

The enzymatic barrier for the permeation of peptide-/protein-type biopharmaceuticals across various absorptive mucosae was recently

Enzymatic Degradation Profiles of Methionine-Enkephalin in Rabbit Mucosal Extracts

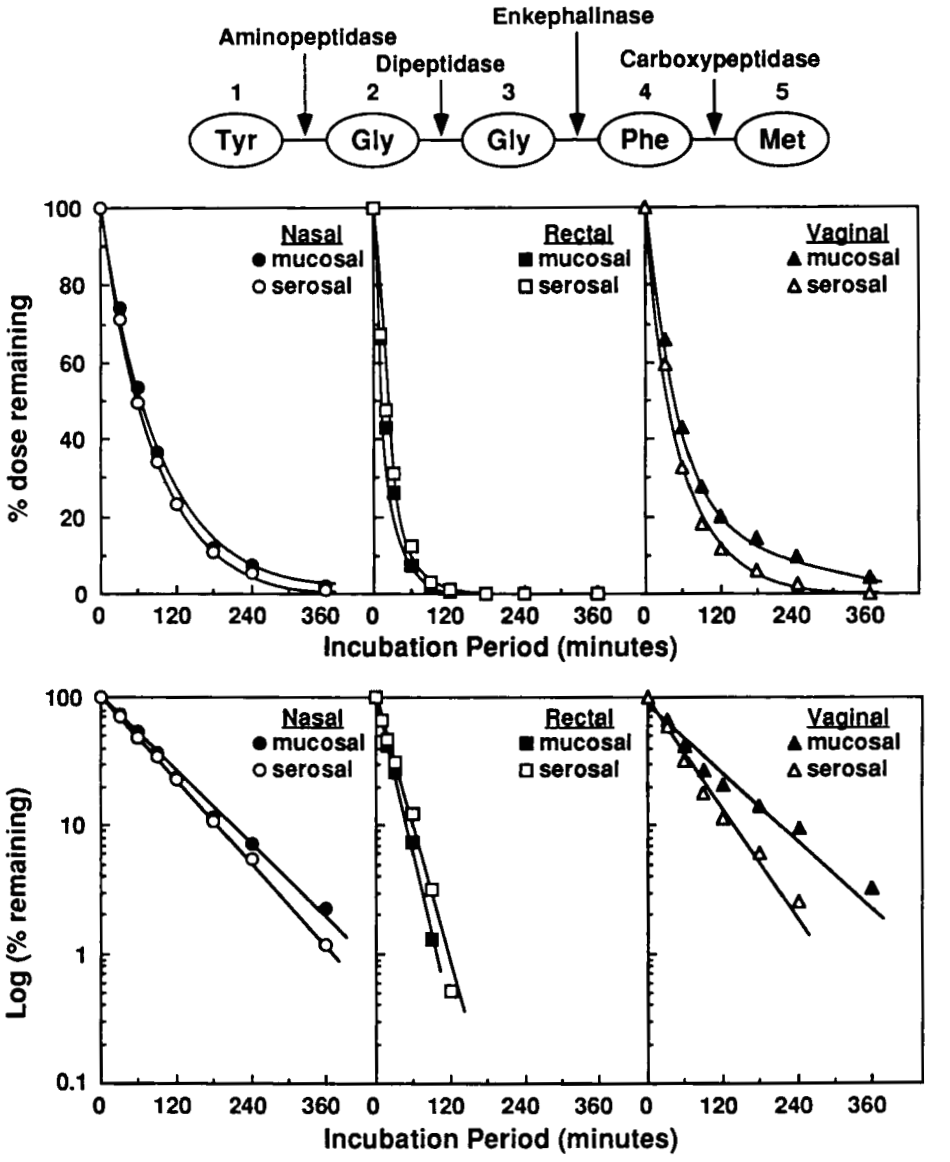

FIGURE 16

(Top) Comparative degradation profiles of methionine-enkephalin incubated in the mucosal and serosal extracts of various rabbit's absorptive mucosae at physiological pH's. (Bottom) First-order kinetic plots for the enzymatic degradation profiles of methionine-enkephalin.

characterized in this laboratory using mucosal extracts, not homogenates, to better simulate in vivo conditions [67]. The extracts were prepared by separately exposing the mucosal and serosal surfaces of various freshly-excised mucosae from albino rabbits to the buffered solution at physiological pH, Leucine- or methionine-enkephalin was then added to these mucosal and serosal extracts and incubated at 37°C. Samples were assayed for intact enkephalin and its degradation products. The enzymatic degradation profiles of methionine-enkephalin are shown in Figures 16 and 17.

The results in Figure 16 suggest that the enzymatic degradation profiles of methionine-enkephalin can be described by a first-order kinetics process. The rate of degradation varies from one mucosa to another (16.2-19.5 x 10^{-3} min^{-1} for rectal, 4.7-7.0 x 10^{-3} min^{-1} for vaginal and 4.6-5.2 x 10^{-3} min^{-1} for nasal). However, the apparent rate constants for the enzymatic degradation of methionine-enkephalin in the mucosal and serosal extracts are not substantially different. On the other hand, hydrolytic degradation, without the involvement of extracted enzymes, is insignificant at these pH levels (with a rate constant of 3.8-5.9 x 10^{-7} min^{-1}).

By monitoring the formation of various degradation products, one may characterize the enzymatic activity in various absorptive mucosae. The results in Figure 17 suggest that aminopeptidase, which is known to be the enzyme responsible for the breakdown of enkephalins by hydrolyzing the Tyr-Gly bond with the formation of tyrosine, has shown greater activity in the rectal mucosa than in the vaginal and nasal mucosae; dipeptidase, whose action is responsible for the formation of a Tyr-Gly fragment by its attack on Gly-Gly bond, has an activity that is substantially greater in the nasal mucosa than in the rectal and vaginal mucosae; enkephalinase, which has been attributed to the formation of a Phe-Met fragment by its attack on Gly-Phe bond, shows an activity pattern similar to that of dipeptidase. On the other hand, the formation profile of phenylalanine resulted from the further degradation of Phe-Met fragment by the action of carboxypeptidase suggests that

Enzymatic Degradation Profiles of Methionine-Enkephalin in Rabbit Mucosal Extracts

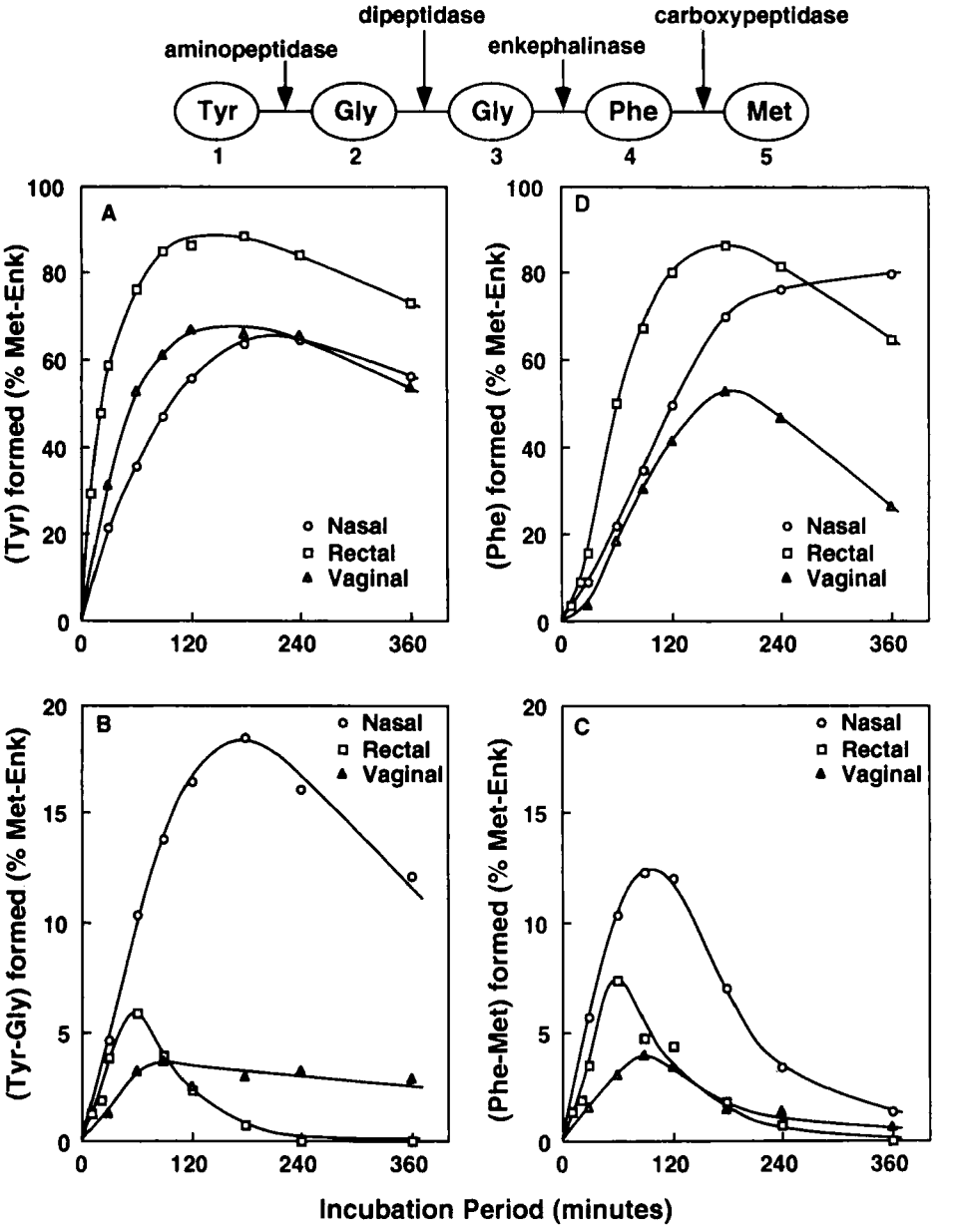

FIGURE 17

Time course for the appearance of some fragments from the enzymatic degradation of methionine-enkephalin incubated in the extracts of various rabbit's absorptive mucosae at physiological pH's.

TABLE 3

Comparison of Rate Constants for Enzymatic Degradation of Enkephalins in Extracts of Various Absorptive Mucosae

Extracts[1]	Physiologic pH	Rate Constant[2] (min^{-1}) x 10^3 Met-Enk	Leu-Enk
Nasal mucosa	6.0	4.81	3.69
Rectal mucosa	7.2	20.64	10.77
Vaginal mucosa	8.0	5.42	4.02

(1) Mucosal and serosal extracts of freshly-excised mucosae from New Zealand white rabbits (n=6 for Met-Enk and n=3 for Leu-Enk).

(2) Mean values of first-order degradation rate contant [n=12 for methionine-enkephalin (Met-Enk) and n=6 for leucine-enkephalin (Leu-Enk)].

carboxypeptidase activity is greater in the rectal mucosa than in the nasal and vaginal mucosae.

Studies were also conducted to investigate the enzymatic degradation kinetics of leucine-enkephalin, another biologically- occurring brain analgesic pentapeptide with the methionine at position 5 in the methionine-enkephalin replaced by leucine. The results indicated that leucine-enkephalin is also rapidly degraded in the extracts of various absorptive mucosae and also follows a first-order kinetic process [68], with the rate constants lower than those for methionine-enkephalin, especially in the rectal mucosa extract (Table 3).

Efforts were extended in this laboratory to investigate effective means to stabilize enkephalins in the extracts of various absorptive mucosae by

adding an antibacterial agent, such as thimerosal, a proteolytic enzyme inhibitor, such as amastatin [69], and a chelating agent, such as EDTA [68]. The degradation kinetic profiles outlined in Figure 18 demonstrate that the enzymatic degradation of leucine-enkephalin is progressively retarded with the addition of thimerosal, amastatin, and EDTA. After incorporation of thimerosal (0.01%), the rate constant for enkephalin degradation was reduced by as much as 4 times in the extracts of nasal and rectal mucosae and 9 times in vaginal mucosa extract. After the addition of amastatin (0.1 mM), the stabilizing effect of thimerosal is further enhanced another 2-4 fold. After further addition of EDTA (10 mM), the enzymatic degradation of leucine-enkephalin is substantially minimized in all extracts (from 3.1-12.3 x 10^{-3} min^{-1} to 8.9-11.5 x 10^{-5} min^{-1}). The overall stability of leucine-enkephalin was improved by 35 times in the nasal extract, 82 times in the vaginal extract, and 107 times in the rectal extract. Similar results were also obtained for methionine-enkephalin [70].

The cutaneous metabolism of leucine-enkephalin has also been studied using the homogenates of human epidermis and cultured human keratinocytes [71]. The results indicated that both homogenates produce similar Km values, apparent first-order rate constants, and metabolite profiles, and homogenates of foreskin, breast skin and cloned cells yield comparable aminopeptidase activities. Furthermore, it was found that in skin homogenates, at least two types of aminopeptidase have been assessed to be responsible for the metabolism of N-terminal amino acid, successively, to the complete degradation of leucine-enkephalin [62], while endopeptidase activity is negligibly small. Amastatin and puromycin were observed to produce the highest inhibitory effect on the cutaneous metabolism of leucine-enkephalin in skin homogenates but only slightly effective in stabilizing leucine-enkephalin during skin permeation studies. The results suggested that proteolytic enzyme activities encountered by peptides and proteins during the course of transdermal delivery are complex, which are different from those

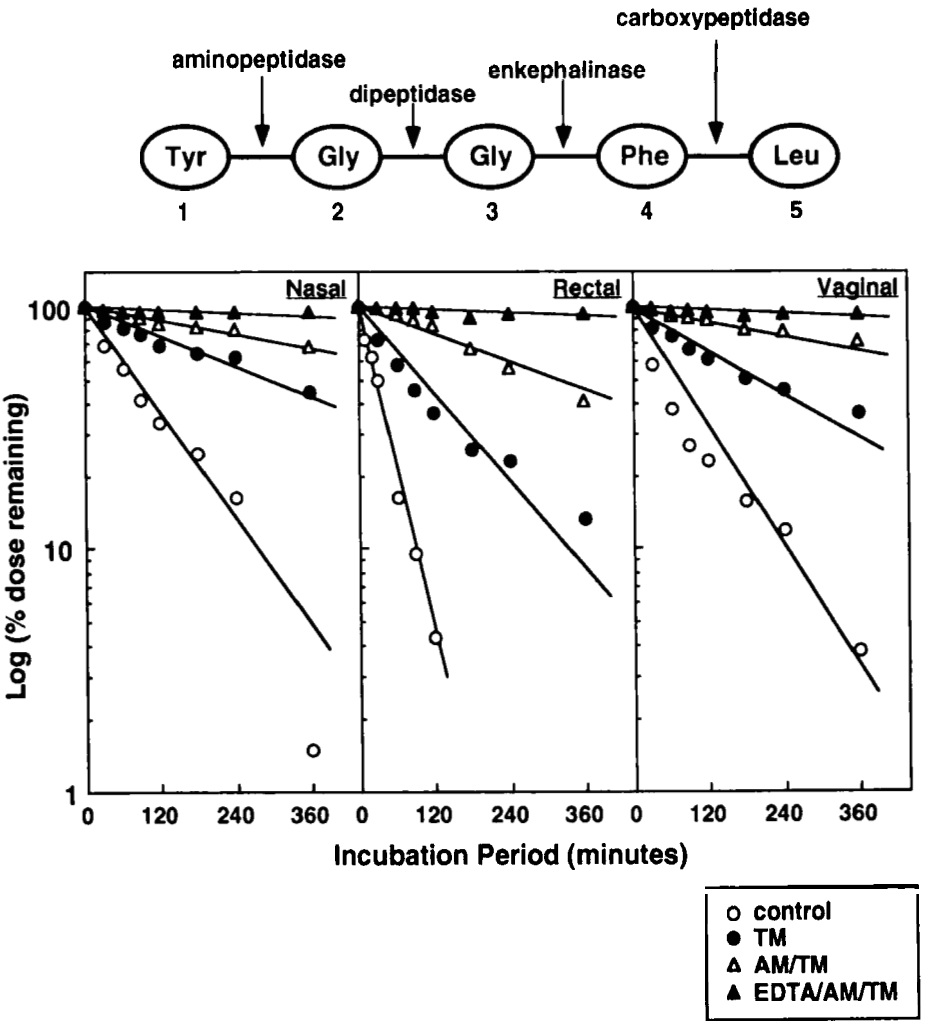

FIGURE 18

Effect of thimerosal (TM) with amastatin (AM) and ethylenediaminetetraacetate (EDTA) on the first-order degradation kinetic profiles of leucine-enkephalin in the extracts of various rabbit's absorptive mucosae at physiological pH's.

TABLE 4

Steady-State Rate of Transmucosal Permeation of Enkephalins

Mucosa[1]	Transmucosal Permeation Rate[2] ($\mu g/cm^2$ per hr ± SEM) Leu-Enkephalin	Met-Enkephalin
Nasal	24.13 (0.14)	8.28 (1.88)
Rectal	0.31 (0.05)	1.43 (0.01)
Vaginal	4.00 (0.80)	2.88 (1.47)

(1) Excised freshly from New Zealand white rabbits (n = 3).

(2) Both donor and receptor solutions contained an enzyme inhibitor combination: Thimerosal (0.01%), Amastatin (0.1 mM), and EDTA (10 mM).

data generated from the enzymatic degradation study conducted in skin homogenates [62].

Using the results obtained in enzyme inhibition studies, a permeation medium was developed, in which enkephalins have the maximal biochemical and biophysical stabilities, for studying the transmucosal permeation kinetics of leucine- and methionine-enkephalins [68, 72]. The results in Table 4 indicated that nasal and vaginal mucosae are approximately 3- and 2-fold more permeable to leucine-enkephalin than to methionine-enkephalin. On the other hand, the rate of permeation across the rectal mucosa is extremely low for leucine-enkephalin, which is 4-5 times lower than the permeation for methionine-enkephalin. For both enkephalins, the rate of transmucosal permeation showed the rank order: nasal > > vaginal > > rectal mucosa. The observation is in good agreement with the transmucosal permeation rate profile of mannitol, a hydrophilic marker, reported earlier in the literature [1].

Further studies demonstrated that the transmucosal permeation of enkephalins can be significantly enhanced by absorption promotors, such as bile salts and dihydrofasidates (DHF). For example, the rate of transmucosal permeation for leucine-enkephalin has been enhanced by phosphate-DHF as much as 28 times for rectal mucosa and 11 times for vaginal mucosa but only 1.5 times for nasal mucosa [68].

In summary, for the successful systemic delivery of peptide-/protein-type biopharmaceuticals through various noninvasive routes of administration, it requires a thorough investigation of the mechanisms and pathways for their biophysicochemical degradation, the addition of various enzyme and/or microbial inhibitors, alone or in combination, to improve their biophysical and biochemical stabilities, as well as the incorporation of absorption promotors to enhance their absorption and systemic bioavailability [72].

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