RESEARCH ARTICLE

Pharmacokinetics and biodistribution of topical phosphosulindac and its metabolites in mice and a mouse model for chemotherapy-induced peripheral neuropathy

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ABSTRACT

Background: Our purpose was to determine the pharmacokinetics and biodistribution of phosphosulindac and its metabolites when applied to mouse skin. Phosphosulindac differs from sulindac by adding a diethyl-phospho-butane moiety, which enhances efficacy and safety. In preclinical studies, phosphosulindac has anti-cancer and anti-inflammatory properties and prevents and reverses chemotherapy-induced peripheral neuropathy

Methods: Phosphosulindac gel was applied topically to the hind paws of normal mice and those with chemotherapy-induced peripheral neuropathy. Samples from the paw skin, paw muscle, leg muscle, sciatic nerve, dorsal root ganglia and blood, obtained at various time points, were assayed using HPLC for phosphosulindac, and its metabolites (sulindac, sulindac sulfide, sulindac sulfone).

Results: Topically applied phosphosulindac was detected in paw skin, paw and adjacent leg muscles where it reached its T_{max} in 0.5 h. Smaller amounts of phosphosulindac were detected in sciatic nerve ($T_{max} = 3$ h) and dorsal root ganglia (at 24 h). Phosphosulindac was not found in blood. Absorption of phosphosulindac was concentration-dependent and pH-sensitive. Its metabolites were detected in paw skin, vicinal muscles, sciatic nerve, and blood but not in dorsal root ganglia. Topically applied equimolar sulindac was detected in paw skin and muscle and in smaller amounts in leg muscle and sciatic nerve.

Conclusion: Phosphosulindac is absorbed through the paw skin and transported from paw and leg muscle to the sciatic nerve and dorsal root ganglia, its target tissues in normal and chemotherapy-induced peripheral neuropathy mice. In contrast, sulindac is transported through the same tissues as well as by the circulation.

Keywords: Topical application, Preclinical pharmacokinetics, Phosphosulindac, OXT-328, Sulindac, Modified NSAIDs, CIPN

Introduction

Phosphosulindac (PS, OXT-328) is a novel agent belonging to the broad class of modified nonsteroidal anti-inflammatory drugs (NSAIDs). Structurally, PS can be considered a derivative of the conventional NSAID sulindac, which has been covalently bound through a carboxylic ester to a diethyl-phospho-butane moiety. This modification has been considered crucial for its safety, as it removes the carboxylic moiety of sulindac, which is responsible for sulindac's gastroduodenal toxicity. Compared to sulindac, PS displayed enhanced pharmacological efficacy in various systems, in some cases as high as 10-fold[1].

In preclinical studies, PS has shown robust anticancer and anti-inflammatory properties, altering pathways central to cell growth and inflammation[2-6]. Recently, we reported that PS has a strong preventive and therapeutic effect on chemotherapy-induced peripheral neuropathy (CIPN)[7]. This side effect of chemotherapeutic agents can be dose-limiting, negatively affecting the patient's treatment regimen and their quality of life[8]. CIPN affects peripheral nerves, as its clinical manifestations are prominent in hands and feet characterized as a "glove-and-stocking" distribution[8]. In our study, PS was applied topically to the hind paws of mice with CIPN, a site of marked allodynia. PS was dose-dependently efficacious on CIPN induced by three different classes of commonly prescribed chemotherapeutic agents. In each case, PS improved and often normalized mechanical and cold allodynia scores as measured on the hind paws of mice. The mechanism by which PS relieves the neuropathy of CIPN appeared to be the suppression of neuroinflammation through its effect on cytokine expression and macrophage activation[7]. The effect of PS on CIPN did not interfere with the anticancer effect of these chemotherapeutic agents. Interestingly, sulindac, its parent NSAID, was devoid of any effect on CIPN, indicating that the intact PS molecule was required for its anti-CIPN activity[7], in agreement with similar findings in other systems[9,10].

The metabolism of PS, as well as its pharmacokinetics (PK) and biodistribution following its oral administration to mice, have been reported elsewhere[9,11]. PS undergoes reduction and oxidation, yielding PS sulfide and PS sulfone; it is also hydrolyzed, releasing sulindac, which generates sulindac sulfide and sulindac sulfone, all of which are glucuronidated. In mice, oral PS is rapidly absorbed, metabolized, and found in blood and other tissues. The central metabolic transformation of PS is the rapid hydrolysis of its carboxylic ester catalyzed by carboxylesterases, an event that determines its efficacy. In both mouse blood and tissues, the levels of PS were low or undetectable, with the dominant species being its metabolites; of those, sulindac had the highest C_{max}.

Given PS's efficacy in CIPN, we undertook a detailed PK and biodistribution study to map where and when detectable levels of PS and its metabolites appear in various tissues of the mouse after its topical application. Our assumption was that to relieve allodynia, the hallmark of CIPN, PS must reach the affected nerves.

Tissues analyzed included the paw skin and its vicinal leg muscles associated with the site of application; the sciatic nerve and the dorsal root ganglia (DRG), its presumed targets of action; and blood, a potential conduit to nerves.

Materials and Methods

MATERIALS

Phosphosulindac was a gift of Medicon Pharmaceuticals, Inc, Setauket, NY. Sulindac and other reagents used in these studies including the excipients ethyl alcohol (derived from grain), triethanolamine, hydroxypropyl methyl cellulose, xanthan gum, sodium citrate, benzalkonium chloride, citric acid and glycerol, were purchased from Sigma-Aldrich (St. Louis, MO).

PS was formulated in a gel as described[7]; hydroxypropyl-methylcellulose was the gelling agent and xanthan gum the phase stabilizer. The pH of each formulation was adjusted to the desired value using citric acid.

ANIMALS

Adult male C57BL/6J mice, eight weeks of age at the beginning of the experiments and weighing 20–30 g (The Jackson Laboratory, Bar Harbor, ME) were housed in an AAALAC-accredited facility in groups of four. Food and water were available *ad libitum*.

Experiments were performed during the light cycle (7:00 am to 7:00 pm) and animals were euthanized with CO_2 asphyxiation. Studies were approved by the Institutional Animal Care and Use Committees of Stony Brook University and followed the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. Animal studies are reported in compliance with the ARRIVE guidelines[12].

PHARMACOKINETICS/BIODISTRIBUTION IN MICE

Phosphosulindac or sulindac gel, each at various concentrations, was applied topically to each hind paw (50 μl per paw) with gentle rubbing. At the time points 0.5h, 1h, 3h, 6h, 12h, and 24h, mice (n = 5-6 mice/time point) were euthanized with CO_2 inhalation. Blood was drawn immediately after death by cardiac puncture. Tissues, including the sciatic nerve and lumbar DRG bilaterally, were dissected quickly, immediately frozen in liquid nitrogen and stored at -80°C until analyzed.

As previously described[13], each plasma sample was mixed with double volume of acetonitrile and centrifuged at 13,200 rpm for 15 min. Tissue samples were weighed, ddH$_2$O (100-300 μL, depending on tissue weight) was added and they were homogenized. Following addition of acetonitrile (twice the volume of the homogenate), the mixture was sonicated for 10 min, centrifuged at 13,200 rpm for 15 min, and analyzed by HPLC, as reported[13]. The limit of quantitation is 0.1 μM for PS and 0.05 μM for sulindac, sulindac sulfone, and sulindac sulfide.

INDUCTION OF CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY

Chemotherapy-induced peripheral neuropathy was induced in male C57BL/6 mice with paclitaxel

(MilliporeSigma, St. Louis, MO) using established protocols[14-16]. Dosing regiments for paclitaxel as follows: Four i.p. injections of 8 mg/kg paclitaxel every other day, resulting in a cumulative dose of 32 mg/kg. Mechanical allodynia thresholds (paw withdraw thresholds, PWT) were determined using von Frey filaments as described[17].

STATISTICAL ANALYSIS

Pharmacokinetic parameters were calculated by Microsoft Excel and PKSolver. We employed non-compartmental analyses.

Results

PHARMACOKINETICS/BIODISTRIBUTION OF PHOSPHOSLULINDAC 8% TOPICALLY APPLIED TO THE SKIN OF MICE

We studied the kinetics and biodistribution of PS when it was applied topically as an 8% pH 9.4 gel to the hind paws of normal mice. To validate our methods, we aimed to measure the amount of PS in the tissues proximal to the site of application as well as tissues more distal with particular attention to the sciatic nerve (SN) and the dorsal root ganglia (DRG) that are altered in CIPN[18].

As shown in Fig. 1A-1B and Table I, PS was detected in paw skin, the site of its application, the muscles underneath the skin (paw muscle), vicinal leg muscles, and the sciatic nerve after it was applied topically to the hind paws of the mice. The concentration of PS progressively decreased from the skin (site of application) to more distal tissues like the SN, as evidenced by the respective values of both C_{max} (from 126.3 ± 4.8 µM to 2.0 ± 0.7 µM) and AUC_{0-24h} (from 918.4 µM·h to 17.0 µM·h). PS was detected in DRG only after 24h at a low concentration of 0.9 µM. However, no PS was detected in the systemic circulation. The T_{max} of PS was the same in all tissues (0.5 h) except for SN, which showed a prolonged T_{max} (3 h). Another interesting feature is the difference in $T_{1/2}$ of the skin, the muscles, and SN, which varied within a wide range (9.6 - 63.7 h); the $T_{1/2}$ of DRG was more prolonged but could not be determined with reasonable accuracy.
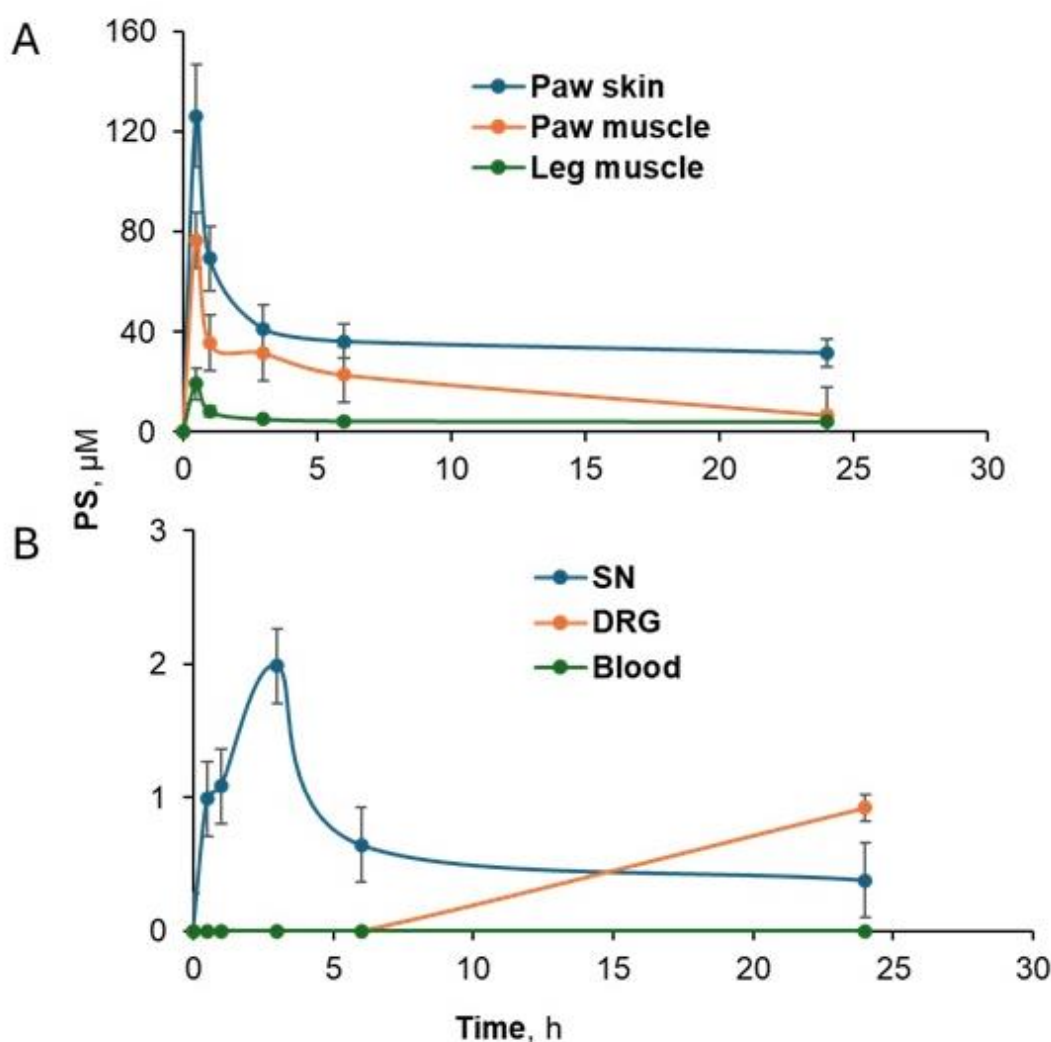

Figure 1. Kinetics and biodistribution of topical PS gel. PS was applied as an 8% pH 9.4 gel gel to the hind paw of normal mice. Concentration of PS progressively decreased from the skin (site of application) to more distal tissues like the sciatic nerve and dorsal root ganglia. (A) Note the Tmax (0.5 h) is the same in the paw skin, paw muscle and leg muscle. (B) PS took longer to reach Tmax in the SN (3 h) and DRG. Intact PS was not detected in the blood.

Table I. PK parameters of PS in mouse tissue and peripheral blood in mice treated with 8% PS at pH9.4

Tissue	C_{max}, µM mean ±SEM	T_{max}, h	$T_{1/2}$, h	AUC_{0-24h}, µM·h
Paw skin	126.3 ± 4.8	0.5	63.7	918.4
Paw muscle	76.6 ± 4.3	0.5	9.6	461.6
Leg muscle	19.2 ± 3.0	0.5	81.8	114.9
SN	2.0 ± 0.7	3	11.3	17.0
DRG	-[a]	-[a]	--[a]	-[a]
Blood	-[a]	-[a]	--[a]	-[a]

[a], cannot be determined. N =5-8 mice/time point.

We detected three metabolites of PS: sulindac, sulindac sulfone, and sulindac sulfide (Figure 2A-D and Table II) in the tissues and the circulation. However, no glucuronidated metabolites were detected and all three appeared in circulation (Fig. 2B, 2C and 2D)[11]. Sulindac was the dominant metabolite, with sulindac sulfide and sulindac sulfone levels being <20% of those of sulindac. However, sulindac sulfide was only detected in the circulation (Fig. 2D). When comparing Cmax data from Tables I and II, sulindac levels were about 1/3 of those of PS in all tissues except the sciatic nerve (where it was greater than 50%). No sulindac was detected in DRG.
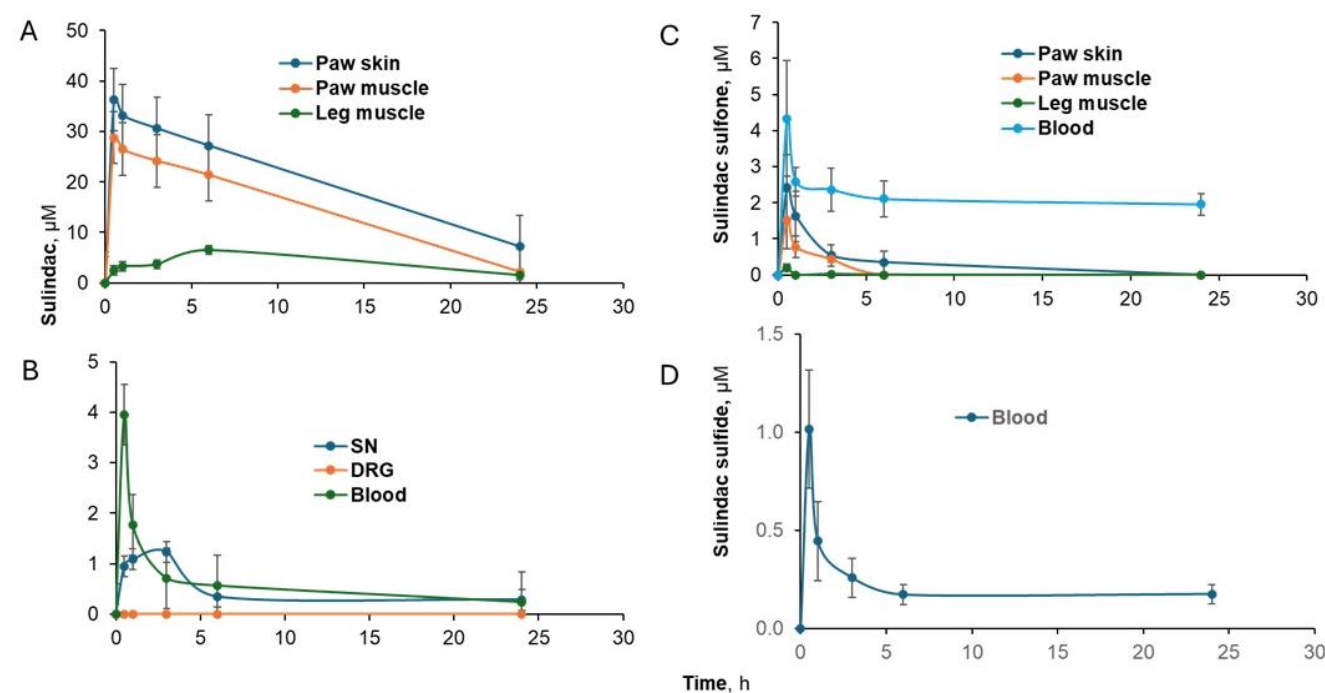

Figure 2. Kinetics and biodistribution of PS metabolites. PS was applied as an 8% pH 9.4 gel gel to the hind paw of normal mice. (A) The main metabolite of PS, sulindac is found in the Paw skin, paw and leg muscle near the site of application. (B) Lower amounts of sulindac were found in the SN and blood but not the DRG. (C) Sulindac sulfone was found in the blood, paw skin, paw muscle and in lesser amounts in the leg muscle. (D) Finally, sulindac sulfide was only found in blood.

Table II. PK parameters of sulindac in mouse tissue and peripheral blood in mice treated with 8% PS at pH 9.4

Tissue	C_{max}, µM mean ±SEM	T_{max}, h	$T_{1/2}$, h	AUC_{0-24h}, µM·h
Paw Skin	36.3 ± 3.6	0.5	9.8	486.9
Paw muscle	28.8 ± 3.4	0.5	5.9	352.5
Leg muscle	6.5 ± 1.9	6	--[a]	97.3
SN	1.2 ± 0.2	3	14.0	11.1
DRG	-[a]	-[a]	-[a]	-[a]
Blood	4.0 ± 1.0	0.5	13.7	14.0

[a], cannot be determined. N =5-8 mice/time point.

Having detected PS and sulindac in SN tissue, we compared the levels of PS and its metabolites in the proximal and distal half of the sciatic nerve of mice 30 min after its application to the hind paw (Table III). The two values were strikingly different, with those of the distal half being 18.9-fold higher than those of its proximal half (17 ± 5.1 µM vs. 0.9 ± 0.3 µM; Table 3). Also shown in Table III, the concentration of the three metabolites of PS (sulindac, sulindac sulfide, and sulindac sulfone) was also higher in the distal half compared to the proximal half (4.5-8.5-fold higher).

Table III. PS and its metabolites in the proximal and distal half of the sciatic nerve in mice treated with 8% PS at pH 9.4

	Distal	Proximal*	Fold difference
	µM, mean ± SEM		
PS	17.0 ± 5.1	0.9 ± 0.3	18.9[a]
Sulindac	3.4 ± 1.1	0.4 ± 0.03	8.5[a]
Sulindac sulfide	3.5 ± 1.0	0	--
Sulindac sulfone	0.9 ± 0.2	0.2 ± 0.08	4.5[a]

[a]All differences between proximal and distal values are statistically significant (p<0.001).
N=8 mice/group

EFFECT OF PS DOSE ON PHARMACOKINETICS/BIO-DISTRIBUTION

Given the dose dependence of the effect of PS in reversing mechanical allodynia in CIPN[7], we investigated the effect of increasing doses of PS on the PK/biodistribution of PS in areas such as the site of its application, contiguous (paw muscle) or vicinal (leg muscle) tissues as well as the sciatic nerve and blood.

Mice were treated with 1.2%, 5%, and 8% PS pH 7.5 gel, in which PS was administered topically to their hind paws, and their tissues were harvested at the indicated time points. As shown in Fig.3A-D and Table IV, PS was detected in paw skin, its application site, the muscles underneath the skin (paw muscle), leg muscles, and the sciatic nerve in a dose-dependent manner. Minimal PS levels were detected in DRG (data not shown) and no PS was detected in the circulation.
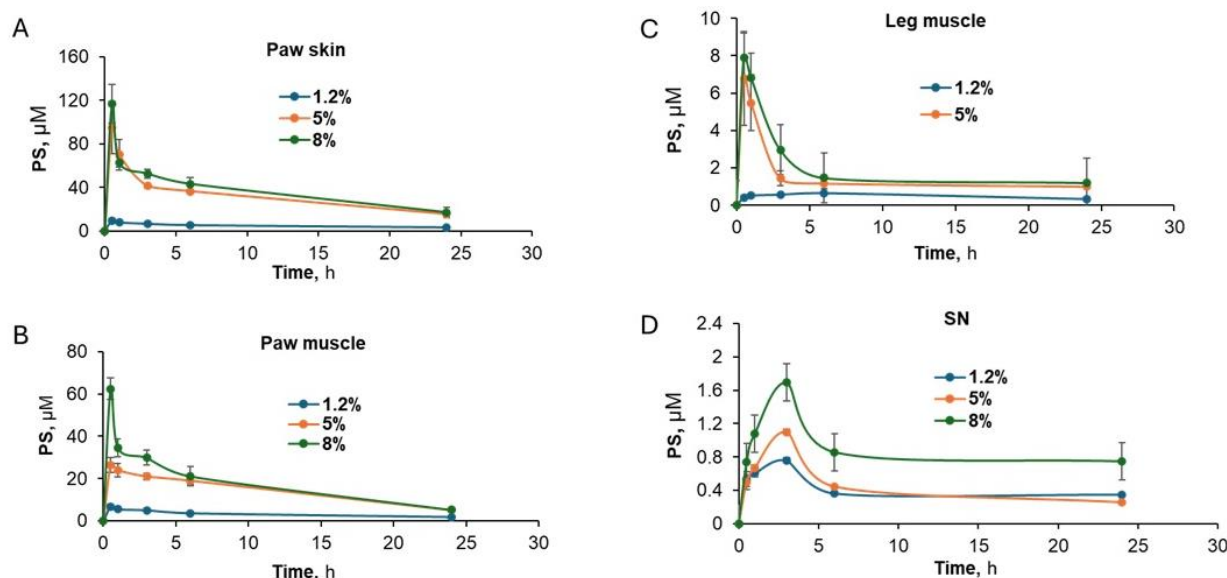

Figure 3. **Effect of concentration on PS kinetics and biodistribution.** The appearance of PS at the site of application (A) paw skin, (B) paw muscle, (C) leg muscle and (D) sciatic nerve was directly proportional to the concentration of PS administered to the hind paw. The further the tissue from the site of application, the lower the levels of measured PS. No PS was detected in the circulation.

Table IV. PK parameters of different PS concentrations in mice treated with different concentrations of PS at pH 7.5

Tissue	C_{max}, µM	T_{max}, h	$T_{1/2}$, h	AUC_{0-24h}, µM·h
	mean ± SEM			
Paw skin				
1.2%	9.8 ± 2.3	0.5	21.9	117.9
5%	94.9 ± 4.9	0.5	14.7	760.7
8%	116.9 ± 4.8	0.5	13.1	877.2
Paw muscle				
1.2%	6.6 ± 1.9	0.5	15.8	79.0
5%	26.3 ± 3.3	0.5	10.2	342.1
8%	62.5 ± 4.1	0.5	8.5	418.6
Leg Muscle				
1.2%	0.6 ± 0.4	6	--[a]	12.0
5%	6.9 ± 2.1	0.5	49.2	35.1
8%	7.9 ± 2.0	0.5	10.2	46.1
SN				
1.2%	0.6 ± 0.2	3	27.7	9.9
5%	1.1 ± 0.1	3	12.2	10.9
8%	1.7 ± 0.5	3	24.7	21.7

[a], cannot be determined. N =5-8 mice/time point

We also detected the three metabolites (sulindac, sulindac sulfone, and sulindac sulfide) for all PS doses administered. As expected, sulindac (Fig. 4A -E) was the most abundant metabolite and it was present in all tissues including blood with higher concentrations detected in mice treated with 5% and 8% PS gels as compared to 1.2% PS (Fig. 4A-E and Table V). However, there were similar sulindac levels in all tissues treated with 5% and 8% PS gels. The other two metabolites, sulindac sulfone and sulindac sulfide, were only detected in blood (Fig. 5A-B) in a dose dependent manner and showed minimal levels in other tissue (data not shown).
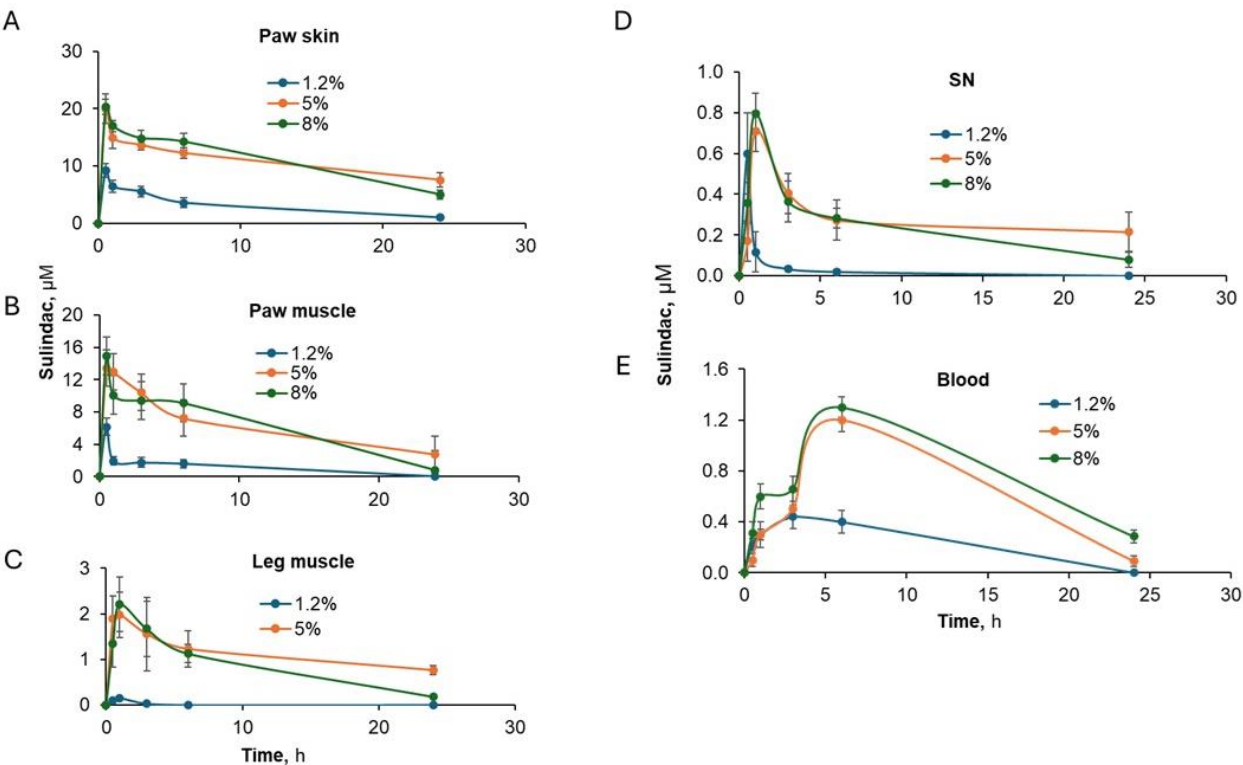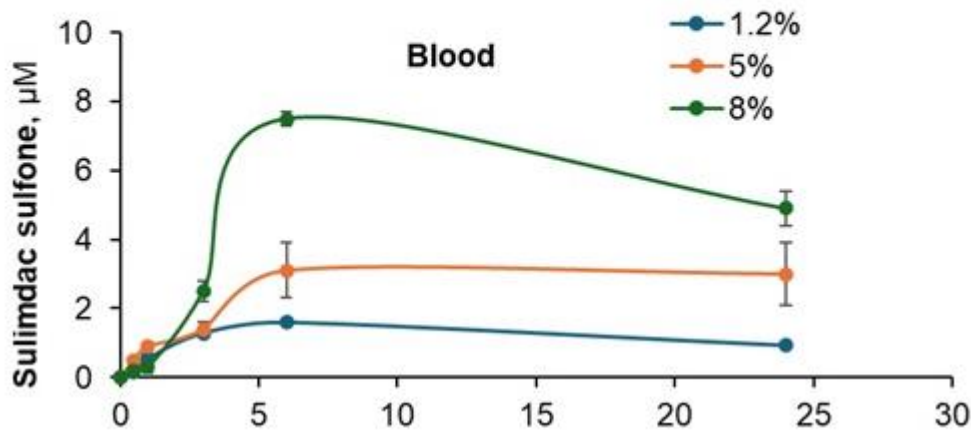

Figure 4. Effect of PS concentration on the kinetics of its main metabolite, sulindac. When PS is administered topically to the paw skin in concentrations from 1.2 %, 5% and 8% pH 7.4, the main metabolite, sulindac can be detected in (A) paw skin, (B) paw muscle, (C) leg muscle, (D) SN and (E) blood. The amount of sulindac is directly proportional to the amount of PS administered. Please note in all tissues the small difference in PS levels when 8% or 5% PS is administered. This may indicate that the enzymes which metabolize PS are at saturation.

Table V. PK parameters of sulindac in mice treated with different concentrations of PS at pH7.5

Tissue	C_{max}, μM mean $\pm$SEM	T_{max}, h	$T_{1/2}$, h	AUC_{0-24h}, μM·h
Paw skin				
1.2%	9.1 $\pm$ 2.2	0.5	8.7	73.5
5%	19.9 $\pm$ 3.2	0.5	25.1	259.2
8%	20.3 $\pm$ 3.0	0.5	13.1	263.9
Paw muscle				
1.2%	6.2 $\pm$ 1.8	0.5	3.9	27.2
5%	13.5 $\pm$ 2.6	0.5	10.4	149.2
8%	14.9 $\pm$ 2.7	0.5	5.5	146.8
Leg Muscle				
1.2%	0.1 $\pm$ 0.03	0.5	1.5	0.1
5%	2.0 $\pm$ 0.6	1	22.1	26.9
8%	2.2 $\pm$ 0.5	1	6.5	22.5
SN				
1.2%	0.6 $\pm$ 0.1	0.5	2.0	0.6
5%	0.7 $\pm$ 0.3	1	28.6	6.8
8%	0.8 $\pm$ 0.3	1	9.7	5.8
Blood				
1.2%	0.4 $\pm$ 0.08	3	-[a]	2.1
5%	1.2 $\pm$ 0.2	6	-[a]	13.8
8%	1.3 $\pm$ 0.3	6	-[a]	18.8

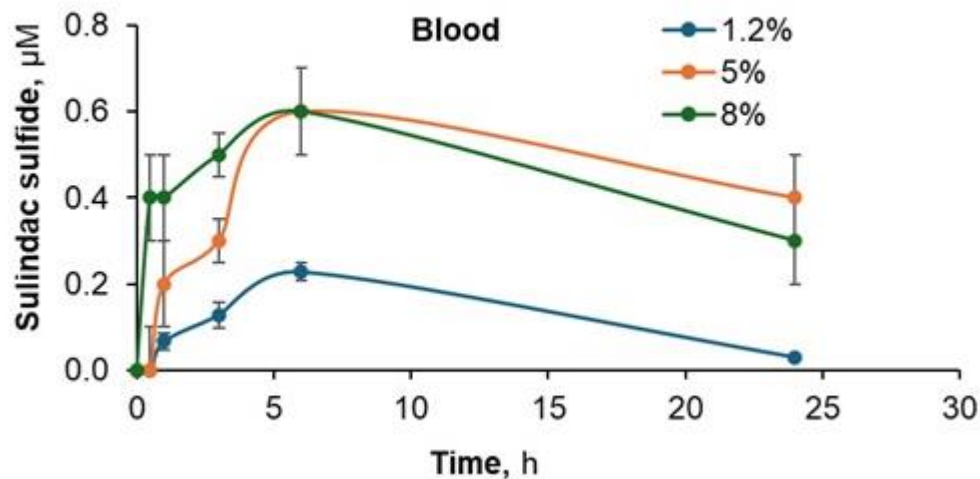[a], cannot be determined. N =5-8 mice/time point

Figure 5. Effect of PS concentration on its other metabolites, sulindac sulfone and sulindac sulfide. (A) Sulindac sulfone was only detected in blood after topical application to the hind paw of mice. The amount of sulindac sulfone is directly proportional to the concentration of PS in the applied gel. (B) Sulindac sulfide was also only found in blood after topical application of PS. Note the crossover of 8% and 5% gels indicating that the enzymes which metabolize PS to sulindac sulfide are being saturated with reactants.

pH EFFECT ON PK/BIODISTRIBUTION OF PS AND ITS EFFICACY ON MECHANICAL ALLODYNIA IN PACLITAXEL INDUCED CIPN

To explore the effect of pH on the efficacy of PS in mechanical allodynia, we compared the efficacy of a 3% gel of PS at pH 7.5 and pH 9.4, as shown in Figure 6A. Higher doses may mask the effect of pH since PS was proved effective from 1.2% to 8%[7]. We have reported that PS at both pH levels significantly improved the mechanical allodynia as measured by paw withdrawal threshold from D0=0.71g, to D15, being 1.11g for pH 7.5 and 1.50 g for pH 9.4; p values were <0.03 and <0.005, respectively. However, PS pH 9.4 showed greater efficacy at D15 in reversing mechanical allodynia in CIPN (pH 9.4 vs. pH 7.5, p<0.007).

To explore this, we tested the effect of pH on the biodistribution of PS in CIPN mice. We administered a pH 7.5 and pH 9.4 gel of 8% PS to the hind paws of the two different groups of CIPN mice. Tissues were harvested at the indicated time points as shown in Figure 6B-E. PS was detected in all tissues, including paw skin, the site of its application, the muscles underneath the skin (paw muscle), leg muscles, and the sciatic nerve but not in the circulation (data not shown). As shown in Figures 6B-E, a higher pH showed a mild increase in drug uptake in all tissues.
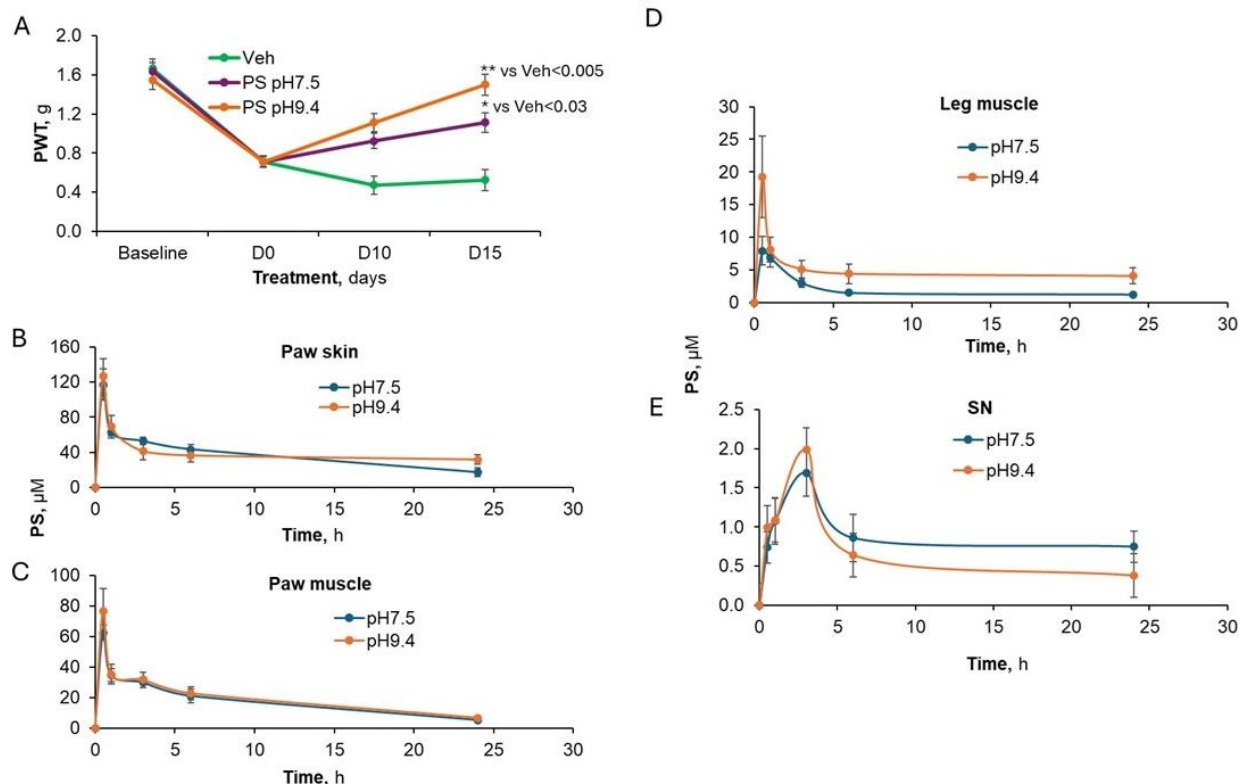
Figure 6. An elevated pH increases the effect of PS on CIPN and its biodistribution. (A) CIPN was induced by repeated injections of paclitaxel which decreased the PWT of all groups. Mice were treated with topical applications of 8% PS at two different pH's or vehicle on D0. The higher pH of 9.4 was more efficacious than the pH 7.5 gel at restoring PWT back to baseline by D15. The p values were (**) <0.005 and (*) <0.03 when compared to vehicle. The effect of pH on PS uptake was measured in (B) paw skin, (C) paw muscle, (D) vicinal leg muscle and (E) sciatic nerve and appears to be only a mild improvement.

We also detected the three metabolites of PS at both pH levels: sulindac, sulindac sulfone, and sulindac sulfide. As shown in figures 7A-E, higher concentrations of sulindac, the metabolite of PS, were detected in all tissues (including blood) in mice treated with the higher pH gel.

As shown in figure 8 A, B respectively, sulindac sulfone and sulindac sulfide were only detected in blood and higher levels were associated with the administration of higher pH 9.4 PS gel.
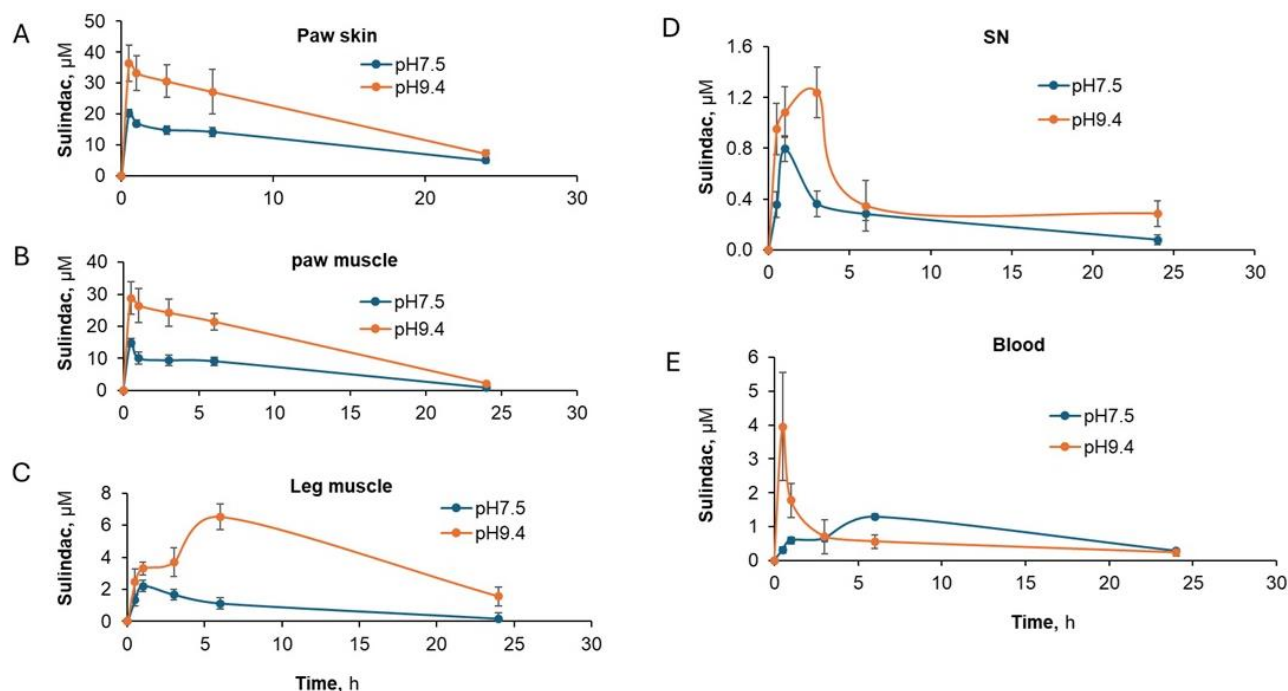

Figure 7. Kinetic and biodistribution of sulindac, the main metabolite of PS, is pH dependent. When tissues from CIPN mice were treated with topically applied 8% PS at two different pH's 7.5 and 9.4, it main metabolite sulindac was measured in (A) paw skin, (B) paw muscle, (C) leg muscle, (D) sciatic nerve and (E) blood. In each tissue, a high pH was associated with a large amount of sulindac.
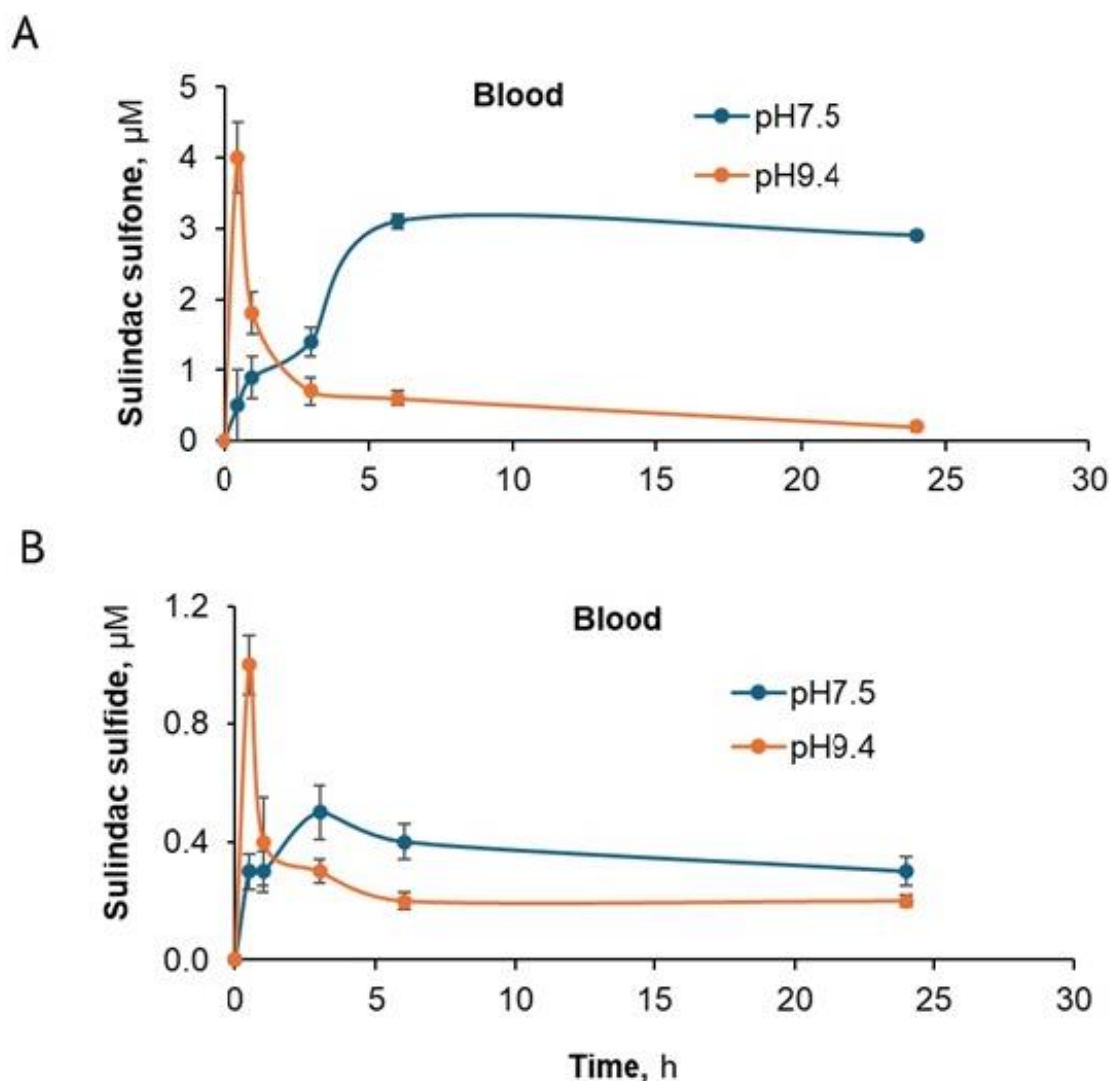
Figure 8. Kinetic profiles of sulindac sulfone and sulindac sulfide in blood are pH sensitive. The PS metabolites of (A) sulindac sulfone and (B) sulindac sulfide are only found in the blood of CIPN mice treated topically with 8% PS gels at pH 7.5 and 9.4. A higher pH was associated with a more rapid appearance of the PS metabolites in blood.

COMPARISON OF PHARMACOKINETICS/BIODISTRIBUTION OF EQUIMOLAR SULINDAC AND PS GELS

As mentioned earlier, sulindac has no efficacy on CIPN, in contrast to PS, when both were applied topically to the hind paws at equimolar concentrations (sulindac 0.7% and PS 1.2% gels)[7]. We performed a PK/biodistribution study of sulindac in which a 0.7% gel was applied to the hind paws of normal mice. This concentration of sulindac is equimolar to a 1.2% gel of PS and is the highest achievable concentration of sulindac in this formulation. Below, we summarize our findings.

As shown in Table VI, when 0.7% sulindac gel is topically administered to the hind paws of mice, higher levels of sulindac were detected in the paw skin, paw muscle and blood when compared to the amount of PS found in these same tissues of mice treated with equimolar 1.2% PS gel. However, lower levels of sulindac were detected in leg muscle and sciatic nerve of mice treated with equimolar sulindac when compared to PS levels in the same tissues from mice treated with equimolar PS (compared to data in Table IV). Intact sulindac is also found in blood, in contrast to PS which is quickly hydrolyzed by carboxylesterases 1 and 2[10] and the two metabolites of sulindac, sulindac sulfone and sulindac sulfide, were detected in the blood of mice treated with topically applied equimolar sulindac as shown in Table VI.

Table VI. PK parameters of sulindac and its metabolites in mouse tissue and peripheral blood in mice with 0.7% Sulindac

Tissue	C_{max}, μM mean ±SEM	T_{max}, h	$T_{1/2}$, h	AUC_{0-24h}, μM·h
		Sulindac		
Paw skin	35.1 ± 3.6	0.5	13.7	431.0
	[9.8 ± 2.3	0.5	21.9	117.9]*
Paw muscle	11.0 ± 2.4	0.5	46.8	92.1
	[6.6 ± 1.9	0.5	15.8	79.0]*
Leg muscle	0.2 ± 0.05	1	--[a]	0.4
	[0.6 ± 0.4	6	--[a]	12.0]*
SN	0.2 ± 0.04	0.5	2.8	0.6
	[0.6 ± 0.2	3	27.7	9.9]*
Blood	13.6 ± 2.6	1	5.9	50.2
	[--[a]	--[a]	--[a]	--[a]]*
		Sulindac sulfone		
Blood	3.6 ± 1.2	1	35.7	70.7
		Sulindac sulfide		
Blood	5.2 ± 1.6	1	5.5	49.1

[a], cannot be determined. N =5 mice/time point.

*, the corresponding values for phosphosulindac in mice treated with 1.2% PS from Table IV, presented here for ease of comparison.

Discussion

Global cancer incidence is estimated at 19.3 million new cases and 10 million deaths annually[19]. Advances in cancer diagnosis and treatment are expected to increase the number of survivors to 26.1 million by 2040[20]. Many patients will receive chemotherapy agents such as platinum compounds (cisplatin, carboplatin, oxaliplatin), taxanes (paclitaxel, docetaxel), vinca alkaloids (vincristine, vinblastine), and bortezomib. A common off-target effect of these therapies is CIPN, with an incidence ranging from 19% to 85%, depending on the drug and dose[21,22]. Though symptoms vary slightly between agents, CIPN often causes severe, debilitating pain, significantly reducing quality of life and sometimes necessitating dose reduction, treatment delays, or discontinuation of life-prolonging therapy[8].

Currently, no agents are approved for CIPN prevention, and only duloxetine is recommended for treatment, with ASCO guidelines assigning it moderate benefit and low harm based on intermediate-quality evidence[23]. The need for effective therapies remains urgent.

CIPN is associated with the loss of sensory nerve fibers in the skin, including those responding to touch, pain, and temperature[22]. Given the peripheral nature of the damage, topical therapies may offer a targeted, safer alternative. Prior trials using lidocaine or capsaicin patches yielded limited success[24,25]. An effective topical therapy could relieve pain with good tolerability, minimal systemic exposure, and a favorable safety profile. In a preclinical model, we found that topical phosphosulindac both prevented and reversed mechanical and cold allodynia in CIPN without compromising the anticancer efficacy of paclitaxel.

The current study provides the first pharmacokinetic and biodistribution profile of PS when it is applied topically to the paw skin of mice. The findings provide valuable information about its potential therapeutic uses and safety profile. Applying 8% PS gel to the hind paws of mice revealed a noticeable variation in the concentration of the drug, with higher levels observed closer to the application site and lower levels in more distant tissues. Notably, PS was discovered in substantial amounts in the skin and muscles of the paw, with gradually decreasing levels observed in the sciatic nerve and dorsal root ganglia. The T_{max} and $T_{1/2}$ values exhibited substantial variations among tissues, suggesting distinct rates of absorption and metabolism. The prolonged maximum concentration of the drug in the dorsal root ganglia indicates a distinct mechanism of drug transportation to neural tissues, which is likely to involve direct diffusion or uptake through the tissues rather than being distributed through the bloodstream, as indicated by the lack of presence of PS in the blood plasma.

The rapid hydrolysis of PS and its subsequent metabolism are highlighted by the detection of PS metabolites, specifically sulindac, sulindac sulfone, and sulindac sulfide, in the circulation. At the same time, the parent compound, PS, is not detected. Of the PS metabolites analyzed, higher concentrations of sulindac (quantitatively the primary metabolite of PS) were found in the various tissues. This is consistent with previous studies on the metabolic transformation of PS.

To estimate the relative distribution of PS after an administered dose, we examined the AUC_{0-24h} as shown in Table 1 and 2 which represents the amount of phosphosulindac and sulindac measured in paw skin, paw muscle, leg muscle and sciatic nerve. If we assume that the sum of the two substances is equivalent to that of the administered dose of 8% PS, then over half of the administered dose remains on the paw skin as PS or sulindac its main metabolite. Only about a third is transported to the paw muscle and about 9% is measured in the leg muscle. Our estimate from this calculation is that only about 1% of an administered dose is transported to the sciatic nerve. This may imply that the continuous presence on paw skin of PS acts as a depot for drug to

target sensory nerve endings damaged by chemotherapeutic agents and associated with peripheral neuropathy. Also important is the observation that only small amounts of PS reach the sciatic nerve and the dorsal root ganglia. If these tissues are a target for PS in CIPN, then they must be exquisitely sensitive to PS's effect. These data also indicate that further improvements in drug efficacy may be achieved by optimizing its formulation and thus enhancing its delivery to the target neural tissues.

We have also shown that the higher the concentration of PS (5 and 8% verses 1.2%) the higher the drug levels in the sciatic nerve as accompanied by a proportional increase in AUC_{0-24h}. This correlation between dosage and biodistribution is consistent with the therapeutic benefits observed in previous studies on CIPN[7]. Remarkably, the pH of the PS formulation had a significant impact on its biodistribution. Although there was no significant difference in the maximum concentration of PS in paw skin and muscle between pH 7.5 and 9.4 solutions (see Figure 6B-C), the concentration in leg muscle was considerably higher at pH 9.4 (Figure 6D). We also found higher concentrations of PS metabolites in mice treated with 8% PS pH 9.4 indicating that the formulation's pH can influence the substance's ability to enter tissues, be metabolized and appear in the bloodstream. The practical significance of these observations must be tempered by the pH-dependent vulnerability of the PS molecule. Specifically, its carboxylic ester linkage between the sulindac molecule and its diethyl-phospho-butane moiety is more susceptible to being hydrolyzed at high pH, thus inactivating the PS molecule. Thus, the dual effect of high pH on biodistribution and metabolism of PS probably merits further scrutiny in future studies.

When sulindac, the main derivative of PS, was applied directly to the skin, it showed distinct pharmacokinetic and biodistribution characteristics. Although sulindac shares a similar structure with PS, it did not reach similar levels in neural tissues. The presence of the intact compound in the circulation revealed the distinct metabolic pathways and biodistribution profiles of the two compounds. Thus, in contrast to PS, the ineffectiveness of equimolar sulindac in CIPN models may among other factors be a consequence of low delivery to neural tissues in contrast to its higher levels in skin and vicinal muscle tissues and highlights the distinct difference in its therapeutic potential as a treatment for CIPN.

Our results can be summarized as follows. Phosphosulindac is found in all tissues analyzed including paw skin its site of application, vicinal muscles, sciatic nerve and dorsal root ganglia but not the circulation. Like other phospho-NSAIDs, PS is metabolized through the P450 system[26], whose expression can differ between tissues[27]. Its principal metabolite, sulindac is found in all the tissues analyzed including blood but not in dorsal root ganglia. The other metabolite, sulindac sulfone, is found in paw skin, paw muscle and blood. Sulindac sulfide, the active metabolic of sulindac, is only found in blood.

The results of this study support the effectiveness of applying PS to the skin as a specific treatment for conditions such as CIPN, where delivering the medication directly to the peripheral nerves and their superficial nerve endings has a beneficial therapeutic effect. Phosphosulindac's ability to reach therapeutic levels in neural tissues in spite of the near total absence in the circulation makes it a promising candidate for further clinical evaluation as a topically applied treatment/prevention of CIPN. Future research should focus on optimizing the pH, drug concentration and perhaps the formulation to improve therapeutic outcomes while reducing systemic exposure and toxicity.

This extensive study on the pharmacokinetics and biodistribution of dermal applied PS gel in a preclinical model provides a foundation for its potential therapeutic application in human patients, specifically for conditions characterized by peripheral neuroinflammation and pain.

Conclusion

This study presents the first pharmacokinetic and biodistribution analysis of PS, a novel therapeutic candidate for CIPN. Our findings demonstrate that a significant fraction of PS remains at the site of its dermal application and reaches directly contiguous nerves, where it presumably exerts its pharmacological actions in CIPN. While the amounts found in sciatic nerve and dorsal root ganglia are much smaller, they may still be indicative of another possible site of action for PS in CIPN, demonstrating targeted biodistribution to key sites of CIPN pathology. The detection of PS in the various tissues at or near its site of application (4-52 pmol/mg tissue) and more distal neural tissues such as sciatic nerve and dorsal root ganglia provide important mechanistic support for its previously reported in vivo efficacy. It also provides data for dose selection and the rationale for its topical dermal application for treating CIPN in mice. Importantly, our results support the continued development of topical PS as a treatment for human CIPN.

Conflict of Interest Statement:

Data Availability Statement: Research data used in preparation of this manuscript is available on reasonable request to the corresponding author.

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