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Antinociceptive Synergy, Additivity, and Subadditivity With Combinations of

Oral Glucosamine Plus Nonopioid Analgesics in Mice

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Abbreviations: glu, glucosamine(2-Amino-2-deoxy-D-glucose); ibu, ibuprofen; COX,

cyclooxygenase

Abstract

Glucosamine (2-Amino-2-deoxy-D-glucose) and glucosamine-containing products have been reported to have efficacy in the treatment of various musculoskeletal disorders. Glucosamine's efficacy, including reduction of pain, is attributed to disease-modifying properties, specifically to cartilage-rebuilding associated with modulation of IL-1-induced activation of chondrocytes and to inhibition of proinflammatory effects of the NF-κ B pathway. However, glucosamine has not been shown to have direct analgesic activity. We report here that commercial glucosamine (90.4% glucosamine sulfate + 9.6% excipients) administered as the sole agent (up to 500 mg/kg, p.o.) was inactive in the mouse abdominal irritant test, but that certain combinations of glucosamine with nonopioid analgesics at the oral doses and ratios tested resulted in a synergistic (ibuprofen and ketoprofen), additive (diclofenac, indomethacin, naproxen, and piroxicam), or subadditive (aspirin and acetaminophen) antinociceptive interaction. In the specific case of ibuprofen, the racemate (standard ibuprofen) produced dose-related antinociception with $ED50 = 26.1 \pm 3.4$ mg/kg. Combinations containing racemic ibuprofen and glucosamine in greater than 1:1 ratio (glucosamine:ibuprofen) were synergistic in the test (*e.g.*, $ED50 = 11.0 \pm 2.1$ for the 9:1 ratio, $p < 0.01$, ANOVA). Combinations containing glucosamine and ibuprofen (2:1 and 9:1) yielded plasma levels of ibuprofen that were no different from administration of ibuprofen alone. The possibility that combinations containing certain fixed-ratios of glucosamine and certain NSAIDs might enhance pain relief in patients with pain or might achieve acceptable levels of pain relief with lower doses of NSAID (reduced adverse effects) is presently being pursued in clinical trials.

Introduction

Glucosamine (2-Amino-2-deoxy-D-glucose), an amino derivative of glucose, is found abundantly in the human body, particularly in connective and cartilage tissue. It serves as a substrate for biosynthesis of mucupolysaccharides and biopolymers of the articulations and bones and has been reported to facilitate the hexosamine pathway of proteoglycan synthesis (Noyszewski *et al.*, 2001). In recent years, glucosamine has been extensively investigated for use as primary or adjunct therapy in the treatment of osteoarthritis (Gottlieb, 1997; Deal and Moskowitz, 1999; Hochberg, 2001; Towheed *et al.*, 2001). Osteoarthritis, the most common arthritic condition (Lawrence *et al.*, 1989; Felson *et al.*, 1989), is characterized by pain and progressive degeneration of cartilage in synovial joints and vertebrae (Brandt and Slemendra, 1993), leading to significant reduction of mobility and quality of life (Steultjens *et al.*, 2001). Although more definitive clinical trials are needed (da Camara and Dowless, 1998; Barclay *et al.*, 1998), much evidence suggests that glucosamine is effective in osteoarthritis. However, the pain reduction that is associated with the use of glucosamine sulfate (Creamer, 2000) and possibly glucosamine hydrochloride (Houpt *et al.*, 1999) has been attributed to disease-modifying properties rather than to a direct analgesic effect. This prompts the following two questions: does glucosamine possess antinociceptive activity?; does it potentiate the antinociceptive action of other (antiarthritic) compounds? To our knowledge glucosamine has not been tested for analgesic (antinociceptive) activity.

Combining analgesics of proven efficacy is a strategy that is intended to achieve one or more therapeutic goals such as facilitating patient compliance, simplifying prescribing, improving efficacy without increasing adverse effects, or decreasing adverse effects without

loss of efficacy (Raffa, 2001). Most of these combinations result in purely additive analgesic effect. For example, drugs that have *similar joint action* (Tallarida, 2000), such as the two opioid agonists morphine and methadone, generally display an additive interaction (*e.g.*, Taber *et al.*, 1969). However, in certain cases, the combination results in an unexpected greater-than-additive (synergistic) analgesic effect (Bolan *et al.,* 2002). The demonstration of synergistic drug interactions between drugs (*e.g.*, Tallarida *et al.*, 1999), enantiomers of the same drug (*e.g.*, Raffa *et al.*, 1993), or between sites of administration (*e.g.*, Tallarida *et al.*, 1989; Raffa *et al.*, 2000) and the underlying statistical analyses needed for this demonstration have been the focus of our group for several years as summarized in a recent monograph (Tallarida, 2000) and review (Tallarida, 2001). A consistent finding that has emerged from this series of studies is that drug synergism is not an intrinsic property of the constituent drugs. Rather, it depends also on the proportion of each in the combination. Another notable finding is that synergism may occur with combinations in which one of the constituents lacks efficacy as a sole agent. Though rare when the active drug is an analgesic, this situation is especially interesting.

We report here that glucosamine sulfate in doses up to 500 mg/kg p.o. had no antinociceptive effect in the mouse abdominal irritant test when it was administered as the sole agent, but that certain fixed-ratio combinations of glucosamine with certain NSAIDs, but not all, displayed statistically significant antinociceptive synergy. Within the class of NSAIDs, the propionic acid derivatives ibuprofen and ketoprofen were most synergistic when tested in combination with glucosamine. The combination of glucosamine and ibuprofen is presently being evaluated in clinical trials.

Materials and Methods

Animals

Male Swiss-Webster mice (Ace Animals, Inc., Boyertown, PA), 25 – 30 g, were used. They were housed in groups of five at $22 \pm 1^{o}C$ for a minimum of 7 days before testing. The animals were maintained on a 12-h light/dark cycle (lights on at 7 a.m.) and fed mouse chow and water *ad libitum* until 3 h before experimentation began (at noon). All testing was performed in accordance with the recommendations and policies of the National Institutes of Health for the care and use of laboratory animals and was approved by the Temple University Institutional Animal Care and Use Committee. Each animal was tested only once.

Compounds

The following compounds were purchased from Sigma (St. Louis, MO): acetylcholine bromide, acetylsalicylic acid, diclofenac sodium, ibuprofen, indomethacin, ketoprofen, S-(+)-naproxen, and piroxicam. Acetaminophen USP was obtained from Mallinckrodt (St. Louis, MO) and glucosamine (90.4% glucosamine sulfate + 9.6 % excipients) was purchased from Nature's Purest (Los Angeles, CA). All compounds were suspended in distilled water containing 2% by volume of Tween®-80

Abdominal Irritant Test

The procedure was similar to that described by Collier *et al.* (1968) with minor modifications. Groups of 10 mice received by gavage (0.01 ml/g) glucosamine, test compound, or vehicle alone. Additional groups of 10 mice each received one of a series of

combined doses of glucosamine and test compound. After 30 min, each mouse was injected

i.p. (0.25 ml/25 g) with an aqueous solution of acetylcholine bromide (5.5 mg/kg). The

animals were then observed for 10 min for the presence or absence of a characteristic

behavioral response (a wave of constriction and elongation passing caudally along the

abdominal wall, accompanied by a twisting of the trunk and followed by extension of the

hindlimbs) (Collier *et al.*, 1968). Percent antinociception was calculated for each dose based

on the percent of mice displaying no nociceptive response.

Evaluation

Antinociceptive testing consisted of combinations containing glucosamine and one of

several different antinociceptive compounds of proven efficacy. In most experimental

designs the combinations contained fixed-ratio amounts of the constituents. Experiments

designed to (statistically) demonstrate synergism usually require complete dose-response

curves of the active agent alone and that same agent in a fixed-ratio combination with

glucosamine. When preliminary tests showed no evidence of synergism, or in cases where

the experiment was run merely to confirm a related finding, we did not aim to obtain complete

dose-response curves in order to avoid inefficiency and unnecessary animal utilization. All of

the antinociceptive results described here were based on binary responses (quantal dose-

response data) in mice observed for the absence or presence of nociception.

Theory and Statistical Analysis

All dose-response data included at least 10 animals at each dose of the drug or drug

combination tested from which the number protected (absence of nociceptive behavioral

response) was observed, thereby yielding a quantal data set for each test condition. Dose-response data analysis used probit regression from which the value of *ED50* (with 95% confidence limits) could be assessed and this value (*ED50*), for each active agent, formed the basis for its subsequent use in fixed-ratio combinations with glucosamine. The two dose-response regression lines, that of the active agent alone and that of the active agent plus glucosamine, permitted statistical testing (ANOVA) to distinguish between synergism and simple additivity based on the positions of the lines. Additionally, *ED50* values, before and after the addition of glucosamine, were compared from the *t*-distribution. Where differences between the proportions for two groups were needed, the ψ^2 distribution (with Yates correction) was employed. The significance level in all tests was taken to be $p < 0.05$. The term 'additive' effect is unambiguous and based entirely on the potencies of two drugs even when one of them lacks efficacy. In this case, the mathematical definition incorporates the potency of the latter drug as infinite and all of the mathematics and statistics apply as they do to two active drugs. The use of 'additive' in this kind of situation is well established. Details of the calculations are described by Tallarida (2000), and all calculations were carried out with the assistance of the program package, *PharmTool Pro* (The McCary Group, Elkins Park, PA).

Measurement of Ibuprofen Plasma Levels

Twelve groups of 5 mice received by gavage the *ED50* dose of ibuprofen (26.1 mg/kg) as the sole agent or two different fixed-ratio combinations (1:2 and 1:9) with glucosamine administered at the same time. At either 30, 60, 90, or 120 min later, each animal was lightly anesthetized with pentobarbital sodium (0.1 – 0.2 ml, s.c.) and blood was drawn into a

heparinized syringe by direct heart puncture. Each of the five mice in each group provided

0.2 ml of blood to give a pooled total of 1 ml of blood/group. Blood levels of ibuprofen were

quantitated using HPLC with UV detection by National Medical Services (Willow Grove,

PA).

Results

Ibuprofen and glucosamine

Ibuprofen was administered in seven doses as the sole agent and exhibited significant and marked dose-dependency with $ED50 = 26.1 \pm 3.4$ mg/kg, p.o. (**Table 1a**). In contrast, glucosamine sulfate, in doses up to 500 mg/kg, produced no effect, *i.e.*, no animals from at least ten tested at each dose were protected from a behavioral response indicative of nociception. Combinations consisting of glucosamine and ibuprofen would therefore be expected to yield a dose-response relation theoretically identical to that of the active agent alone. When the combination of glucosamine and ibuprofen in a 9:1 ratio was tested the combination also exhibited significant dose-dependency and a pronounced potency enhancement as indicated by the reduced $ED50$ of 11.0 ± 2.1 (**Table 1b**). **Fig. 1** illustrates the probit regression lines for ibuprofen alone and for this combination. The combination regression is significantly displaced (ANOVA, $p < 0.01$) from that of the sole agent, further demonstrating enhanced potency and efficacy that are indicative of synergism for the combination.

The finding of synergism for this fixed-ratio combination prompted additional experiments that employed several different combination ratios. In order to conduct these tests efficiently we used a fixed quantity of ibuprofen equal to its $ED50$ (26.1 mg/kg) along with six different quantities of glucosamine sulfate. As seen in **Table 2**, of the six different combinations two (denoted A and B) produced the expected (additive) response of 50%, whereas each of groups C - F exhibited responses greater than 50%. It is notable that the two additive responses occurred with the lowest proportions of glucosamine (groups A and B). When A and B were combined and C - F combined these two groupings lead to response

proportions 10/20 and 43/50, respectively. The difference between these proportions is highly

significant ($p < 0.01$ from ψ^2 with Yates correction; *see* Tallarida, 2000). It appears that

increasing the dose of glucosamine greater than 52.2 mg/kg did not further increase thew

antinociceptive effect, possibly suggesting a threshold effect. However, our experiments

employed a fixed-ratio because synergism is most often a property of the two chemicals and

the ratio of these and because the use of fixed-ratio provides the design needed in a rigorous

statistical analysis of results. It follows from the results that combinations containing glu:ibu

greater than 1:1 are synergistic in this antinociceptive test.

Tests with other NSAID's

Ketoprofen

Table 3 shows the data obtained from tests with ketoprofen, alone and in a

combination of glucosamine sulfate to ketoprofen of 2.63:1 by weight. Probit regression

revealed a highly significant difference in the two regressions, with *ED50* values of 94.8 ±

30.0 mg/kg for ketoprofen (alone) and 24.2 ± 11.7 mg/kg for ketoprofen in the combination.

Thus, ketoprofen, like ibuprofen, is synergistic with glucosamine in this test of

antinociception.

Indomethacin

Indomethacin (alone) produced dose-response data as follows: 2.5 mg/kg, 4/10; 5.0

(mg/kg), 6/10 and 10.0 (mg/kg), 7/10. Probit regression led to *ED50* = 3.7 mg/kg. When this

quantity, 3.7 mg/kg, was combined with 182.5 mg/kg of glucosamine the result was 5/10

protected *(graph not shown)* which is consistent with simple additivity.

Diclofenac

Diclofenac (0.5 – 5 mg/kg), given alone, gave dose-dependency (data not shown) that yielded an *ED50* = 1.1 mg/kg. When this quantity of diclofenac was administered with 125 mg/kg of glucosamine the protection was only 25% (among 20 animals tested). Doubling this combination dose (2.2, 250) produced protection in only 60% (of 10 animals tested). These results are consistent with a simply additive interaction.

Aspirin

Aspirin (ASA) alone and ASA in a fixed-ratio combination with glucosamine were also tested. Doses of ASA (mg/kg) were 50, 100, 150 and 200. In the combination experiment the respective doses of glucosamine added to ASA were 125, 250, 375 and 500 mg/kg so that the ratio, ASA:Gluc, was maintained at 1.0:2.5. As seen in **Fig. 2** ASA alone exhibited a clear dose-dependency with an *ED50* = 109.2 ± 15 mg/kg. In the combination experiment the efficacy of ASA was reduced to approximately 20% and was essentially flat over the dose range tested, indicating a pronounced antagonism. Because glucosamine, in doses up to 500 mg/kg, is devoid of antinociceptive activity in this test it is expected that an additive combination should produce theoretically coincident dose-response lines. The severe depression of the combination line that occurred was significantly below the ASA dose-response line and, thus, no estimate of *ED50* was possible for this combination. These results show that glucosamine strongly antagonizes the antinociceptive activity of aspirin in this test.

Naproxen

Naproxen (7.5 – 120 mg/kg, p.o.) induced dose-dependent antinociception (data not shown) with an *ED50* = 36.1 ± 14 mg/kg. Regression analysis of the combination of naproxen with glucosamine in a fixed ratio of 3:1 (glucosamine:naproxen) produced a line that did not differ significantly (F = 0.13) from that of naproxen alone, thereby indicating simple additivity for this combination.

Piroxicam

Piroxicam alone (0.75 – 3.0 mg/kg, p.o.) induced dose-dependent antinociception with *ED50* = 1.5 ± 0.1 mg/kg (data not shown). The *ED50* dose of piroxicam (1.5 mg/kg) was then tested in combination with 75 mg/kg of glucosamine (50:1 ratio glucosamine:piroxicam). None of the animals that received this combination reached the criterion for antinociception, *i.e.*, 0/10 response proportion. When the glucosamine concentration was increased to 150 mg/kg with the same amount of piroxicam (100:1 ratio glucosamine:piroxicam), the response proportion was 1/10. These results represent a marked subadditivity, *i.e.*, antagonism by glucosamine of the antinociceptive action of piroxicam.

Acetaminophen

The results of experiments with acetaminophen, alone and in combination with different fixed ratios of glucosamine, are shown in **Fig. 3**. It is seen that APAP alone shows significant dose-dependency with an *ED50* = 146.1 ± 21.4 mg/kg. The addition of glucosamine, in all fixed-ratio combinations tested, diminished the APAP response. When the APAP: Glu ratio was 4:1, the diminution in response was evident from the downwardly translated dose-response line. When the APAP proportion of the combination was further

reduced (1:1 and 1:4, as shown in **Fig. 3**) the antinociception was virtually abolished. These results show that glucosamine strongly antagonizes the antinociceptive activity of acetaminophen in this test.

Plasma levels of ibuprofen

A blood sample (0.2 ml) was obtained from each mouse and pooled with the four others in its group (1.0 ml total) at 30, 60, 90, and 120 min following oral administration of the *ED50* dose of ibuprofen as the sole agent, and in two different fixed-ratio combinations (2:1 and 9:1) with glucosamine administered at the same time. The plasma level of ibuprofen at each time point was essentially unaffected by co-administration with glucosamine (**Fig 4**). The plasma level of ibuprofen rose rapidly after administration to 35 – 40 µg/ml at 30 min, then progressively declined with no significant difference with or without glucosamine (2:1 or 9:1 fixed ratio) over the subsequent 100 min.

Discussion

The pain that is commonly associated with damaged tissue that accompanies arthritis, sports injuries, and other conditions often originates from multiple sources and can involve multiple 'types'. For example, in the case of osteoarthritis of a joint, potential sources of pain include the synovial membrane, joint capsule, periarticular ligaments or muscle, periosteum, and subchondral bone (Brandt and Slemenda, 1993). It also involves multiple types, both inflammatory and non-inflammatory, and the signal is transmitted via multiple pain transmission pathways (such as Aδ- and C-fiber primary afferents). Because of the multifaceted nature of this type of pain, treatment can often benefit from, or necessitate, a

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combination of mechanistic approaches. Combining analgesic agents to achieve this goal is a rational therapy for pain (Raffa, 2001). The potential benefits of analgesic combinations are straightforward, such as the opportunity for better patient compliance, enhanced efficacy or efficacy against a broader spectrum of pain types. Combinations also provide the potential for a reduction of unwanted effects. Combination analgesic therapy has been recommended as part of a comprehensive pain-management paradigm by the World Health Organization (WHO) (Schug *et al.*, 1990), the American Pain Society (1999) and the American College of Rheumatology (2000).

Although the potential advantages of combinations are well known, actual evidence of synergy (antinociception or analgesia statistically greater than the expected additivity) have rarely been documented. An exception is the recently approved combination of tramadol with acetaminophen (Tallarida and Raffa, 1996). Part of the problem has been the relative lack of methodology to test for synergy with efficiency or with any degree of statistical rigor. With new methodology (*e.g.*, Tallarida, 1992; Tallarida *et al.*, 1997a,b; 1999) this has been resolved to a great extent (Tallarida, 2000). The analysis covers the condition in which one agent does not demonstrate antinociceptive activity when adminstered as the sole agent, such as glucosamine in the present situation.

Each of the NSAIDs tested in the present study (diclofenac, ibuprofen, indomethacin, ketoprofen, piroxicam, and naproxen) and acetaminophen induced antinociception in the mouse abdominal irritant test. Glucosamine sulfate was inactive up to the highest dose tested (500 mg/kg). Fixed-ratio combinations of glucosamine with the NSAIDs and acetaminophen were evaluated for an additive effect (*i.e.*, the same as the analgesic administered as the sole agent) or an effect suggestive of an interaction.

The significant antinociceptive synergism that was elicited by fixed-ratio combinations of glucosamine + ibuprofen and glucosamine + ketoprofen stand in stark contrast to the additive or subadditive interactions produced by combinations of glucosamine with the other analgesics tested. Moreover, the synergism was shown to depend strongly on the proportions of the agents in the combination, thereby emphasizing that a synergistic interaction is not merely a property of the constituents; it also depends on their relative concentrations. Whereas the chief mechanistic characteristic shared by the NSAID group is inhibition of prostaglandin biosynthesis, there are other mechanisms associated with the individual agents to varying degrees. Each agent has its own pharmacodynamic and pharmacokinetic characteristics. The most obvious attribute that distinguishes ibuprofen and ketoprofen from the other analgesics we tested is that each is a propionic acid derivative (α-Methyl-4-(2-methylpropyl)benzeneacetic acid and 3-Benzoyl-α-methyl-benzeneacetic acid, respectively). However the other propionic acid derivative naproxen ((αS)-6-Methoxy-α-methyl-2-naphthaleneacetic acid), did not synergize with glucosamine (at least at the ratios tested). Indomethacin, which is an indole acetic acid derivative (1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid), diclofenac, which is a phenylacetic acid derivative (2-[2,6-Dichlorophenyl)amino]benzeneacetic acid), and piroxicam, which is an enolic acid (4-Hydroxy-2-methyl-N-2-pyridinyl-2H-1,2-benzothiazine-3-carboxamide), exhibited only additive antinociception with glucosamine. The salicylic acid derivative aspirin (2-(Acetyloxy)benzoic acid) and the non-NSAID p-aminophenyl derivative acetaminophen (N-(4-hydroxyphenyl)acetamide) demonstrated subadditive effects with glucosamine. Hence, a simple chemical structure-based classification of synergy is not possible at present. Likewise, although the doses of glucosamine which affected the

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analgesics fell within a relatively narrow range despite the variation in *ED50* values, it is difficult to relate this to mechanism since the outcomes were different (synergistic with two analgesics, additive with four analgesics, and subadditive with the two other analgesics).

The widespread usage of ibuprofen guided its selection for our more detailed study. Toward that end, our initial focus was on pharmacokinetics, *i.e.*, does the presence of glucosamine significantly alter (increase) the concentration of ibuprofen in the blood and is that the reason for the potency enhancement. That experiment (**Fig 4**) showed virtual identical blood concentration-time relations for the two cases, negating a pharmacokinetic explanation. That finding prompted additional experiments designed to illuminate the mechanism of this drug interaction. In that regard we conducted experiments involving combinations of glucosamine with each of the enantiomers of ibuprofen as a basis for comparing these with the combination containing racemic (standard) ibuprofen. The results of pilot experiments were suggestive of synergy between glucosamine and each of the *R*(–) and *S*(+) enantiomers of ibuprofen. These are viewed as preliminary experiments and no statistical significance is attached to these data at this time. Our studies of the enantiomers are, instead, first steps aimed at elucidating the mechanism(s) underlying racemic ibuprofen's synergism with glucosamine. It is interesting to note, however, that both of the enantiomers of ibuprofen were active in the mouse abdominal irritant test and that the *R*(–) enantiomer was more potent than the *S*(+) enantiomer (*ED50* = 18.5 and 48.7 mg/kg p.o., respectively). The enantiomers of ibuprofen differ in terms of their pharmacologic properties. For example, at clinically relevant concentrations, the *S*(+) enantiomer of ibuprofen inhibits cyclooxygenase (COX), whereas the *R*(–) enantiomer is not a COX inhibitor (Evans, 2001). The antinociceptive potency demonstrated by the *R*(–) enantiomer, and its possible synergism with

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glucosamine, further suggest that the synergy is via some mechanism other than COX

inhibition. This might partly explain glucosamine's synergy obtained with ibuprofen (and

ketoprofen), and lack of synergy with the other analgesic compounds we tested. Future

studies of mechanism, already begun, are examining the enantiomers in more detail, including

the 'metabolic inversion' of $R(–)$-ibuprofen to $S(+)$-ibuprofen (Evans, 2001), as well as a

detailed study of ibuprofen pharmacokinetics in synovial fluid.

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Figure Legends

Fig. 1   Probit regressions for ibuprofen alone (*open circles*) and ibuprofen plus glucosamine in a 1:9 ratio (*filled circles*). The dose indicated is that of ibuprofen (mg/kg, p.o.). The right-hand scale shows the percentages corresponding to the probits shown on the left-hand scale. The combination regression is significantly displaced ($p < 0.01$), demonstrating enhanced potency and, thus, synergism for the combination.

Fig. 2   Dose-dependent antinociception produced by aspirin (ASA) in the range 50 – 200 mg/kg p.o. when given alone (*open circles*) and the marked reduction when simultaneously administered with glucosamine in the ratio (Glu:ASA) 2.5:1 (*filled circles*).

Fig. 3   Dose-dependent antinociception produced by acetaminophen (APAP) in the range 62.5 – 250 mg/kg p.o. when administered alone (*open circles*) and when simultaneously administered with glucosamine in the ratio (Glu/APAP) 1:1 (*diamonds*), 1:4 (*filled triangles*), or 4:1 (*filled circles*).

Fig. 4   The figure shows the blood concentration of ibuprofen in mice at four times following administration of its *ED50* dose (26.1 mg/kg, p.o) as the sole agent (*open circles*), and in two different fixed-ratio combinations of 2:1 (filled squares) and 9:1 (filled circles) with glucosamine administered at the same time. Each point (ibuprofen concentration value) is from a sample containing 1.0 ml of blood obtained by pooling 0.2 ml from each of five animals sacrificed at the indicated time following oral dosing.

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Keywords

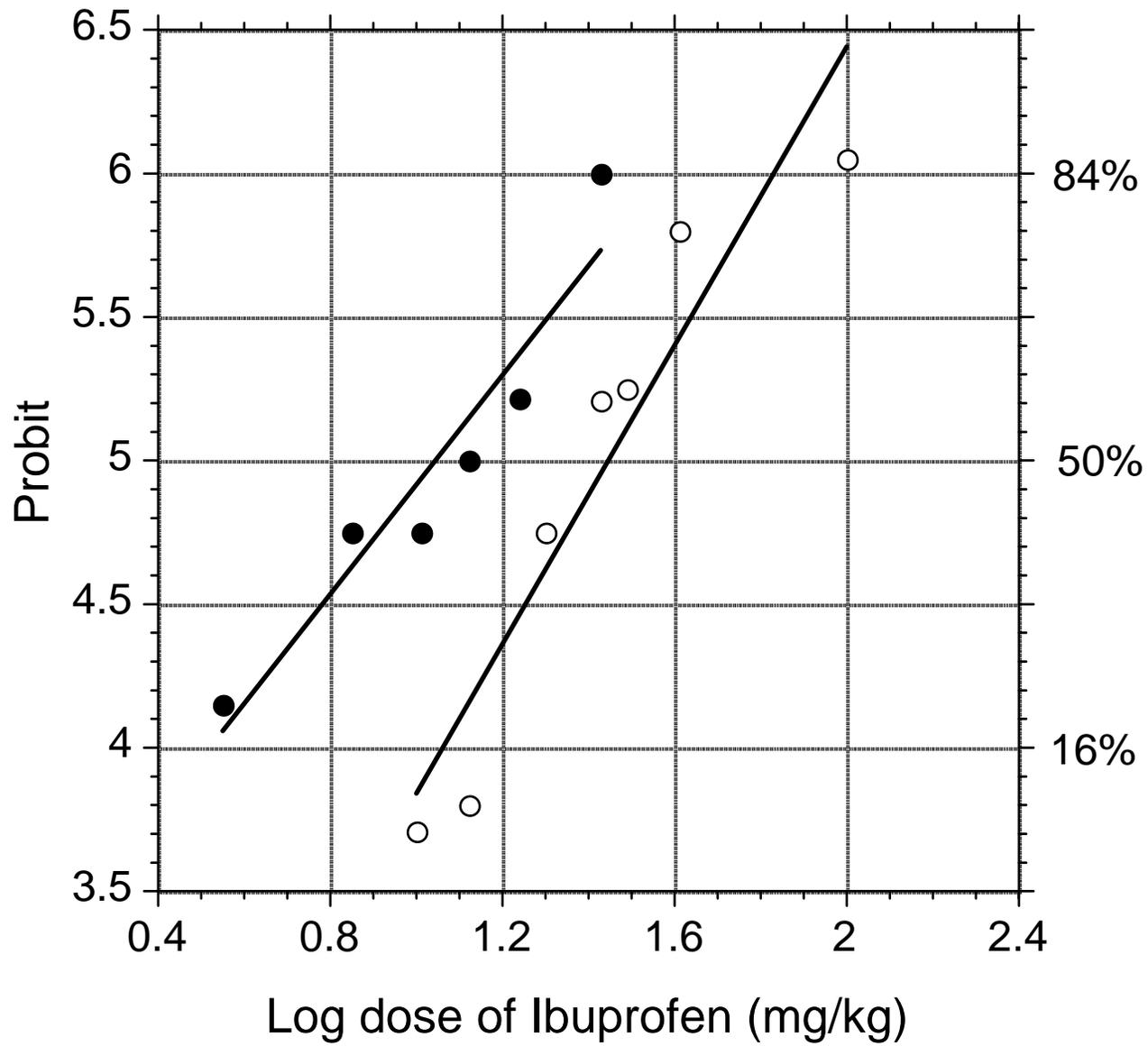Combination, antinociception, synergy, glucosamine, NSAIDs
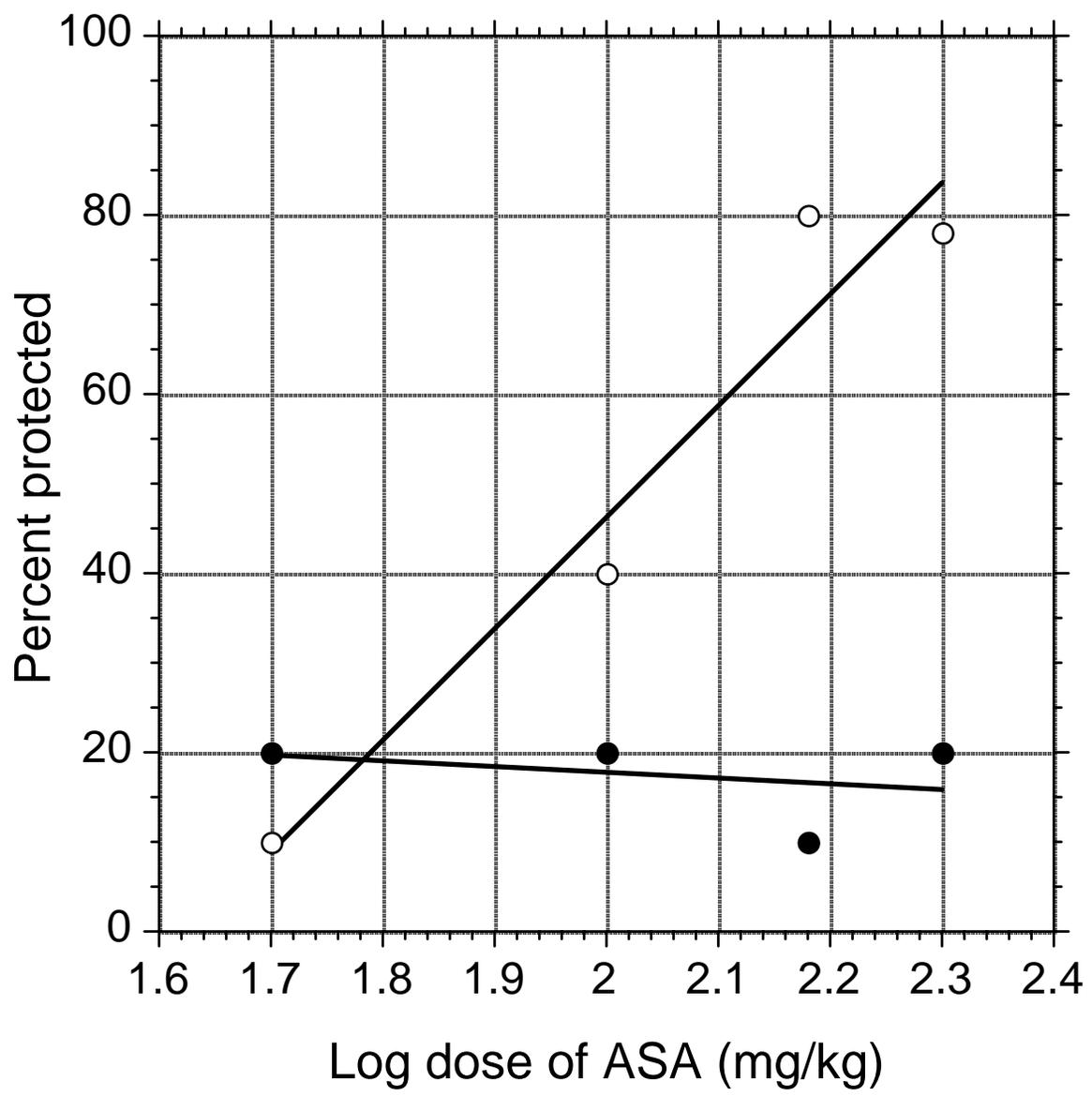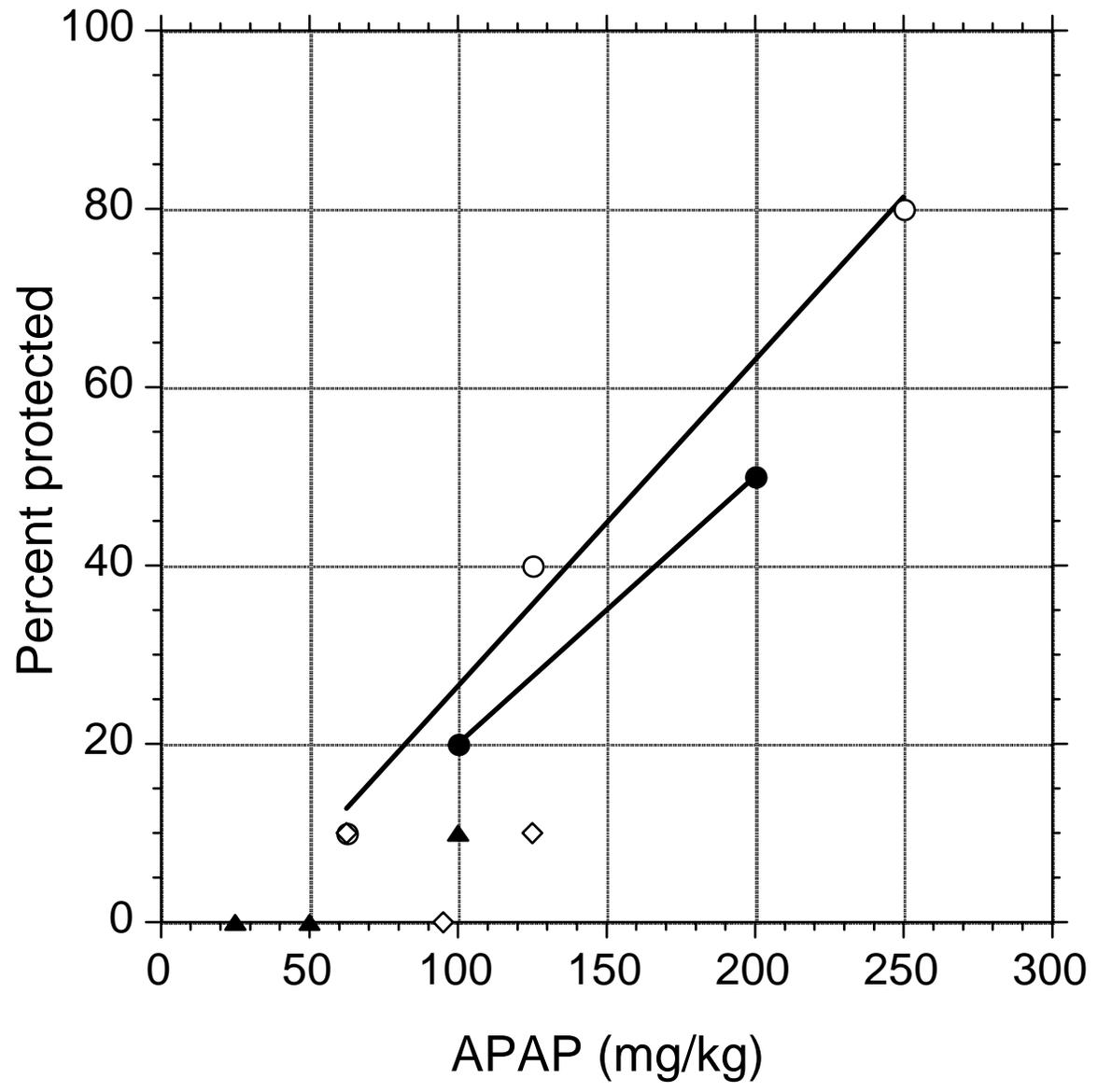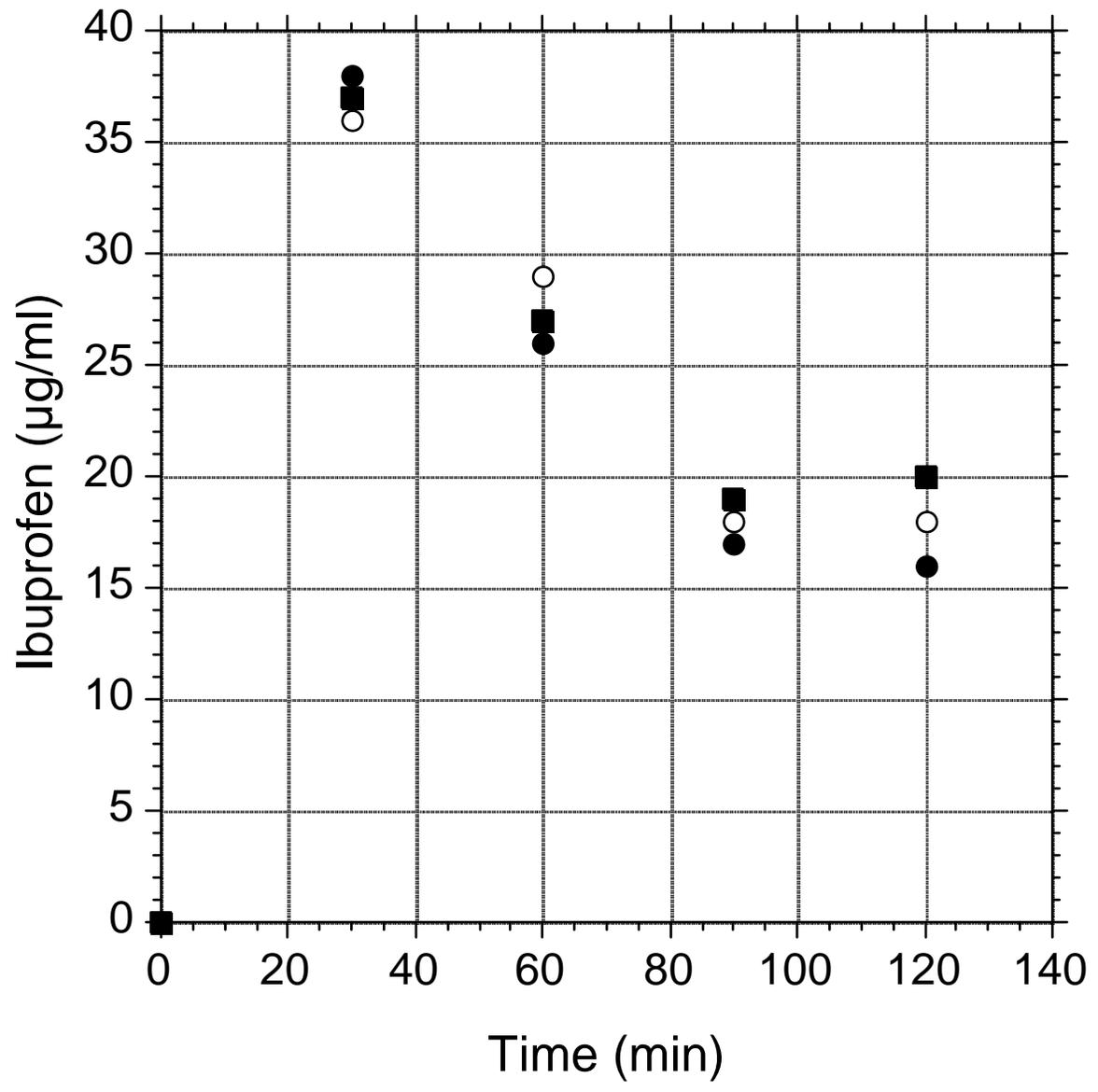
Fig. 1

Fig. 2

Fig. 3

Fig. 4

Table 1

Dose-related antinociception produced by the oral administration of (*RS*)-ibuprofen or the combination of glucosamine sulfate (Glu) plus ibuprofen (Ibu) (weight ratio 9:1) to mice. Antinociception was measured 30 min after dosing and is expressed as the ratio: (number of non-responders)/(number in group). The *ED50* (± 95% confidence interval) was determined from probit regression analysis.

Treatment	Antinociception	*ED50* (mg/kg, p.o.)
a) Ibuprofen (alone)		
10.0	1/10	
13.9	1/10	
20.0	4/10	
27.8	6/10	26.1 ± 3.4
30.0	6/10	
41.7	8/10	
100	9/10	
b) Glu:Ibu (9:1)		
31.2/3.5	2/10	
62.5/7.0	4/10	
93.7/10.4	4/10	11.0 ± 2.1 *
125/13.9	5/10	
154/18.0 (8.6:1)	6/10	
250/27.8	17/20	

* Significantly different from ibuprofen alone ($p < 0.01$, ANOVA).

Table 2

Antinociceptive effect produced by a fixed quantity (26.1 mg/kg, p.o.) of ibuprofen (Ibu)

plus each of six different amounts of glucosamine sulfate (Glu) to mice. Antinociception

was measured 30 min after dosing and is expressed according to: antinociception = (number

of non-responders)/(number in group).

Group	Glu	Ibu	Ratio	Antinociception expected	actual	
A	13.1	26.1	0.5:1	5/10	5/10	10/20
B	26.1	26.1	1:1	5/10	5/10	
C	52.2	26.1	2:1	5/10	8/10	
D	131	26.1	5:1	5/10	9/10	
E	250	27.8	9:1	10/20	17/20	43/50 *
F	500	26.1	19:1	5/10	9/10	

* Significantly different from A and B combined ($p < 0.01$, ψ^2 with Yates correction).

Table 3

Dose-related antinociception produced by the oral administration of ketoprofen or the combination of glucosamine sulfate (Glu) plus ketoprofen (Keto) (weight ratio 2.63:1) to mice. Antinociception was measured 30 min after dosing and is expressed as the ratio: (number of non-responders)/(number in group). The *ED50* ($\pm$ 95% confidence interval) was determined from probit regression analysis.

Treatment	Antinociception	*ED50* (mg/kg, p.o.)
a) Ketoprofen (alone)		
30	2/10	
60	4/10	
120	6/10	94.8 ± 30.0
240	7/10	
b) Glu:Keto (2.63:1)		
62.5/23.8	5/10	
93.2/35.6	6/10	24.2 ± 11.7 *
125/47.5	7/10	

* Significantly different from ketoprofen alone ($p < 0.01$, ANOVA).