Drug and Chemical Toxicology, 28:409–421, 2005

ISSN: 0148-0545 print / 1525-6014 online
DOI: 10.1080/01480540500262805

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Combined Treatment Potentiates the Developmental Toxicity of Ibuprofen and Acetazolamide in Rats

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Aspirin (ASA), an irreversible cyclooxygenase (COX) inhibitor, induces ventricular septal defect (VSD) and diaphragmatic hernia (DH) in rat fetuses when administered on gestation days (GDs) 9–10, a critical period for cardiovascular (CV) and midline development. Evaluation of a spectrum of nonsteroidal antiinflammatory drugs (NSAIDs; reversible COX inhibitors) showed that while some NSAIDs induced VSD in rats, none of the NSAIDs evaluated produced DH. In addition to inhibiting COX, ASA also inhibits carbonic anhydrase. The purpose of this study was to determine whether concurrent inhibition of COX and carbonic anhydrase would produce a teratogenic profile that includes both VSD and DH. To inhibit both COX and carbonic anhydrase, ibuprofen (COX inhibitor) and acetazolamide (carbonic anhydrase inhibitor) were coadministered on GDs 9–10. Groups of 20 female Crl:CD(SD)IGS BR rats were given either 300 mg kg^{-1} day^{-1} ibuprofen, 1000 mg kg^{-1} day^{-1} acetazolamide, or both (combination of ibuprofen and acetazolamide). Fetuses were evaluated on GD 21 for external and visceral development. Ibuprofen induced VSD in 3.7% of fetuses per litter; no defects in appendicular skeletal development were noted. Acetazolamide induced VSD in 5.9% of the fetuses per litter and appendicular defects in 41% of the fetuses per litter. Coadministration of ibuprofen and acetazolamide produced VSD in 18.7% of the fetuses per litter and appendicular defects in 77% of the fetuses per litter; however, there were no DH. Therefore, while concurrent inhibition of COX and carbonic anhydrase did not produce DH, potentiation was noted for the induction of VSD and appendicular anomalies.

Keywords Acetazolamide, Ibuprofen teratology, Rat.

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INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely prescribed drugs and are used for the treatment of several conditions including rheumatoid arthritis, osteoarthritis, and relief from many types of pain. Inhibition of cyclooxygenase (COX) is the common mechanism through which NSAIDs produce their clinical benefits (Vane, 1971). COX exists as two isoforms, COX-1 and COX-2 (Vane et al., 1998). Aspirin, an NSAID that irreversibly inhibits both COX-1 and COX-2 via acetylation, induces ventricular septal defect (VSD), diaphragmatic hernia (DH), and midline defects (MD), such as gastroschisis and omphalocele when administered as a single dose during sensitive periods of development in rats (Gupta et al., 2003; Kimmel et al., 1971). A review of nonaspirin NSAID teratology evaluations in experimental animals suggested the occurrence of a low incidence of VSD, but did not associate NSAIDs with occurrence of DH or MDs (Cook et al., 2002).

The VSDs induced by aspirin (ASA) and other NSAIDs appears to be mediated by COX-1 inhibition (Cappon et al., 2003; Streck et al., 2002). When a series of NSAIDs with different capacities to inhibit COX-1 and COX-2 were administered to pregnant rats during the sensitive period for heart development and midline closure, VSDs were associated with NSAIDs that selectively inhibit COX-1 or have a high ratio of COX-1 to COX-2 inhibition (Cappon et al., 2003). While the findings from this study were consistent with the hypothesis that inhibition of COX-1 is the mechanism by which NSAIDs induce VSDs in rats, the mechanism by which ASA, but not other NSAIDs, produces DH and other MDs is unknown because none of the NSAIDs tested produced an increase in DH or MDs (Cappon et al., 2003). In addition to the inhibition of COX, ASA has been shown to inhibit carbonic anhydrase (Kivilaakso, 1982). Inhibition of carbonic anhydrase alone does not cause DH or MDs (Maren et al., 1993; Scott et al., 1981). However, it is possible that concurrent inhibition of carbonic anhydrase and COX may underlie ASA-induced MDs and DH. The purpose of this study was to test the hypothesis that concurrent inhibition of COX-1 and carbonic anhydrase will produce a teratogenic profile similar to ASA, that is, induce both VSD and DH. Concurrent inhibition of COX and carbonic anhydrase was accomplished by coadministering ibuprofen (COX inhibitor) and acetazolamide (carbonic anhydrase inhibitor) during the sensitive period for midline closure and cardiovascular development (GDs 9–10) (DeSesso, 1997).

MATERIALS AND METHODS

Test Article and Treatment Regimen

Groups of 20 pregnant rats were given 300 mg kg^{-1} day^{-1} ibuprofen (orally), 1000 mg kg^{-1} day^{-1} acetazolamide (SC injection), or both on GDs 9

and 10. GDs 9–10 has been shown to be a sensitive period for midline closure and cardiovascular development (DeSesso, 1997). Acetazolamide or 0.5% methylcellulose was administered by subcutaneous injection twice daily with an interdose interval of 7 h at a dose volume of 5 mL/kg body weight. Ibuprofen or 0.5% methylcellulose was administered by oral gavage once daily at a dose volume of 10 mL/kg body weight. A similar group of 20 control rats was administered 0.5% methylcellulose by the same routes and regimen as the combination treated group. The two groups given a single treatment (ibuprofen or acetazolamide) were administered a vehicle dose by the same route (i.e., the ibuprofen group was given twice daily injections of vehicle, and the acetazolamide group was given a single gavage administration of vehicle).

The dose levels of ibuprofen and acetazolamide were based on previous studies. Ibuprofen administered on GDs 9 and 10 at a dose of 300 mg kg^{-1} day^{-1} was shown to be a maximum tolerated dose and also induced an increase in VSD (Cappon et al., 2003). Acetazolamide has been shown to produce a range of teratogenic effects that primarily result in malformations of the distal postaxial part of the right forelimb but also include hydrocephaly, VSDs, missing ribs, anophthalmia, hydronephrosis, and polydactyly and syndactyly of the hindlimbs (Maren, 1965; Scott, 1970; Wilson et al., 1968). The dose and route of administration shown to be most effective in generating these effects was 1000 mg kg^{-1} day^{-1} given twice daily (with a 7-h interdose interval) by subcutaneous injection. Doses were adjusted according to daily body weight and were administered at approximately the same time each day.

Animals and Housing

One hundred timed-pregnant female Crl:CD(SD)IGS BR rats (Charles River Breeding Laboratories, Kingston, NY, USA) between 10 and 12 weeks of age and at least 225 g at the time of mating were used for this study. GD 0 was defined as the day that a sperm plug was observed. The rats were housed individually in polycarbonate cages with contact bedding in the form of heat-treated hard wood chips. Room lighting was set for a 12-h light/dark cycle. Certified Rodent Diet 5002 (PMI Feeds, Inc.) and drinking water were provided ad libitum. The animal care and experimental procedures of this study were conducted in compliance with the U.S. Animal Welfare Act and performed in accordance with the standards of the Institute of Laboratory Animal Resources (ILAR) Guide, 1996. The Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International has accredited the Pfizer facility in which this study was conducted.

Maternal Observations and Measurements

All animals were observed at least twice daily for morbidity and mortality during the study. Body weights and feed consumption were measured on the

day the animals arrived, and on GDs 8, 9, 10, 11, 12, 15, 18, and 21. Cesarean sections were performed on GD 21. The abdominal, thoracic, and pelvic viscera were examined grossly. The number of corpora lutea on each ovary was recorded. The number, type, and location of implantation sites were recorded. Viable fetuses were removed from the uteri and weighed. A detailed external examination of each fetus was conducted. The fetuses were euthanized by oral ingestion of sodium pentobarbital and were examined for internal anomalies, using a modification of the Stuckhardt and Poppe method (Stuckhardt and Poppe, 1984). The brain and ventricles were examined for dilations or abnormalities by making a single cut along the coronal suture of the cranium. Fetal skeletons were not examined. The nomenclature for description of developmental anomalies was adapted from the International Federation of Teratology Societies (IFTS) glossary (Wise et al., 1997).

Statistical Evaluation

Body weight, corrected maternal body weight gain, and feed consumption data were analyzed using t-tests with each treated group compared to the control group isolated from all of the other treated groups. Cesarean section observations (numbers of corpora lutea, viable fetuses, and the percentages of pre- and postimplantation loss) were analyzed using the Wilcoxon rank sum test with each treated group compared to the control group isolated from all of the other treated groups. Fetal data were analyzed based on litter means. Fetal weight was analyzed using a one-way ANOVA. If the ANOVA was significant ($p \leq 0.05$), Tukey multiple comparison test was used to compare treatment groups. Incidences of specific malformations were analyzed using the

Table 1: Maternal body weights (g) during gestation.

GD	Control	Ibuprofen	Acetazolamide	Ibuprofen + acetazolamide
9	281 ± 14	285 ± 10	280 ± 22	281 ± 11
10	285 ± 19	278 ± 11	259 ± 17**	254 ± 10**
11	285 ± 17	261 ± 11**	251 ± 18**	245 ± 9**
12	291 ± 14	262 ± 16**	257 ± 19**	242 ± 10**
15	313 ± 17	288 ± 24**	298 ± 17**	263 ± 23**
18	354 ± 21	329 ± 39*	336 ± 19**	310 ± 19**
21	403 ± 26	378 ± 43	389 ± 27	355 ± 28**
Corrected BW gain	18.4 ± 14	−3.8 ± 33*	16.2 ± 19	−4.0 ± 17**

Data presented as mean ± standard deviation. Corrected body weight gain equals the body weight gain from GD 9 to 21 less the gravid uterine weight.
*$p \leq 0.05$.
**$p \leq 0.01$ vs. control group.

Table 2: Feed consumption (g/day) during gestation.

GD	Control	Ibuprofen	Acetazolamide	Ibuprofen + acetazolamide
9	21 ± 4	22 ± 3	22 ± 2	21 ± 3
10	22 ± 5	11 ± 9**	7 ± 5**	2 ± 2**
11	17 ± 7	4 ± 5**	3 ± 3**	1 ± 1**
12	22 ± 4	11 ± 7**	13 ± 5**	6 ± 3**
15	70 ± 10	60 ± 21	72 ± 9	41 ± 16**
18	76 ± 12	73 ± 23	81 ± 12	75 ± 8
21	73 ± 14	72 ± 11	77 ± 10	76 ± 8

Data presented as mean ± standard deviation.
*$p \leq 0.05$.
**$p \leq 0.01$ vs. control group.

Kruskal–Wallis test. If the Kruskal–Wallis test was significant ($p \leq 0.05$), Dunn multiple comparison test was used to compare treatment groups.

RESULTS

Administration of ibuprofen, acetazolamide, or the combination of ibuprofen and acetazolamide on GDs 9 and 10 reduced maternal body weight and feed consumption (Tables 1 and 2). The greatest reduction in body weight

Table 3: Cesarean section observations.

	Control	Ibuprofen	Acetazolamide	Ibuprofen + acetazolamide
Number of rats per group	20	19	20	20
Number pregnant dams	20	18	20	18
Corpora lutea	16.1 ± 2.1	15.3 ± 3.4	15.1 ± 1.9	14.7 ± 2.3
Implantation sites	14.3 ± 1.8	13.6 ± 2.1	14.1 ± 1.8	13.8 ± 1.9
Preimplantation loss (%)[a]	10.2 ± 10.7	8.9 ± 14	6.0 ± 7.7	5.4 ± 8.8
Early resorptions	0.6 ± 0.7	0.3 ± 0.6	0.6 ± 1.2	1.8 ± 1.8*
Late resorptions	0	0	0.1 ± 0.2	0
Postimplantation loss (%)[b]	4.0 ± 5.2	2.0 ± 4.2	5.3 ± 9.9	13.0 ± 12.8*
Viable fetuses	13.8 ± 2.0	13.3 ± 2.1	13.4 ± 2.4	12.1 ± 2.5*
Sex ratio (male/female)	0.5 ± 0.2	0.5 ± 0.2	0.5 ± 0.1	0.5 ± 0.2

Data presented as mean litter values ± SD.
[a]Preimplantation loss (% per litter) = ((no. corpora lutea − no. of implantation sites)/no. corpora lutea) × 100.
[b]Postimplantation loss (% per litter) = ((no. implantation sites − no. of live fetuses)/no. of implantation sites) × 100.
*$p \leq 0.05$.
**$p < 0.01$ vs. control group.

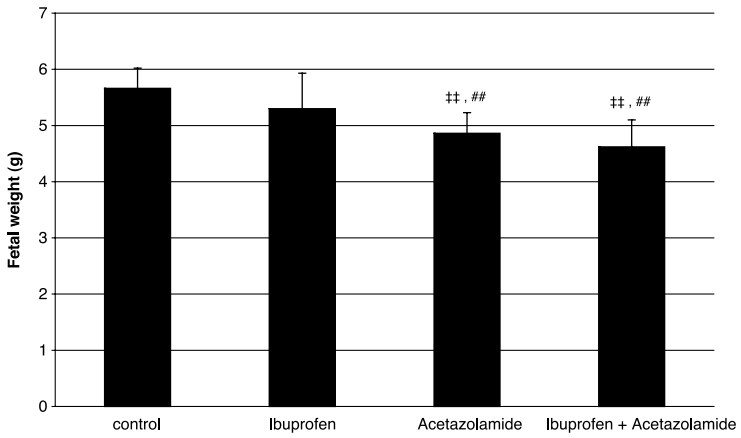

Figure 1: Acetazolamide significantly reduced fetal weights. Combining ibuprofen slightly increased the decrement in fetal weight, although there were no statistical differences between the acetazolamide and acetazolamide + ibuprofen groups. $^{‡‡}p \leq 0.01$ vs. control; $^{\#\#}p \leq 0.01$ vs. Ibuprofen.

occurred on GD 12 (0.90 ×, 0.88 ×, and 0.83 × control for the ibuprofen, acetazolamide, and combination group, respectively). Although dams treated with the combination of ibuprofen and acetazolamide had a greater decrement in maternal body weight than for either of the agents administered individually (not statistically compared), the corrected body weight gain

Table 4: Fetal external findings.

	Control	Ibuprofen	Acetazolamide	Ibuprofen + acetazolamide
Number fetuses evaluated (litters)	275 (20)	240 (18)	268 (20)	229 (19)
Appendicular anomalies				
Hemimelia	0	0	12.8 (29)	53.5 (120)*,**
Amelia	0	0	0.4 (1)	0.5 (1)
Ectrodactyly	0	0	40.1 (105)*	72.8 (165)*
Polydactyly	0	0	0	3 (3)
Adactyly	0	0	0.4 (1)	8.8 (20)*,**
Apodia	0	0	1.6 (4)	1.8 (4)
Brachydactyly	0	0	0.7 (2)	0
Other external				
Cleft palate	0	0	0	0.4 (1)
Cleft lip	0	0	0	0.4 (1)
Mandibular agnathia	0	0	0	0.4 (1)
Anus imperforate	0	0	0	0.4 (1)
Thoracogastroschisis	0	0	0	0.4 (1)
Omphalocele	0	0.5 (1)	0	0
Acaudate	0	0	0	1.4 (3)
Short tail	0	0	0.6 (2)	3.9 (9)

Data presented as the percent per litter (number of fetuses) with finding. In many instances, a single fetus had multiple findings.
*$p \leq 0.01$ vs. control.
**$p \leq 0.01$ vs. acetazolamide.

(body weight gain from GD 9 to 21 minus the gravid uterine weight) showed the same deficit for the ibuprofen alone and combination ibuprofen and acetazolamide groups and was not affected by administration of acetazolamide alone (Table 1).

Administration of either ibuprofen or acetazolamide had no affect on embryo–fetal viability. The numbers of early and late resorptions were comparable to the control group (Table 3). Dams coadministered ibuprofen and acetazolamide had a statistically significant ($p \leq 0.05$) increase in early resorptions that resulted in a subsequent increase in postimplantation loss and a decrease in the number of viable fetuses. Fetal weight was reduced in all treatment groups (0.95 ×, 0.86 ×, and 0.82 × control in the ibuprofen, acetazolamide, and combination groups, respectively), although only the reductions caused by acetazolamide and the combination were statistically significant ($p \leq 0.01$) compared to the control group (Figure 1). The fetal weights of the acetazolamide and combination groups were significantly ($p \leq 0.01$) less than the weight of fetuses from the ibuprofen only group.

Treatment with ibuprofen did not cause an increase in external anomalies (Table 4). Treatment with acetazolamide induced numerous appendicular anomalies, the most common being ectrodactyly (affecting 40.1% fetuses per litter) and hemimelia (affecting 12.8% fetuses per litter). Coadministration of ibuprofen and acetazolamide produced an increase in external anomalies; the

Table 5: Fetal visceral findings.

	Control	Ibuprofen	Acetazolamide	Ibuprofen + acetazolamide
Number fetuses evaluated (litters)	275 (20)	240 (18)	268 (20)	229 (19)
Visceral anomalies				
Diaphragmatic hernia	0	0	0	0
Common truncus	0	0	0	0.3 (1)
Absent innominate artery	0	0.5 (1)	2.5 (7)*	0.9 (2)
Malpositioned subclavian artery	0	0	0	0.9 (2)
Malpositioned carotid artery	0	0	0	0.4 (1)
Interrupted aortic arch	0	0	0.5 (1)	0.4 (1)
Retroesophageal aortic arch	0	0	1.1 (3)	0
Right sided aortic arch	0	0	0.7 (2)	0
Retroesophageal subclavian artery	0	0	0.5 (1)	0
Membranous VSD	0.4 (1)	3.7 (9)	5.9 (15)	18.7 (41)**
Enlarged atrium	0	0	0.7 (2)	0.5 (1)

Data presented as the percent per litter (number of fetuses) with finding. In many instances, a single fetus had multiple findings.
There were no statistical differences between the combined ibuprofen and acetazolamide groups and the independent treatment groups.
*$p \leq 0.05$.
**$p \leq 0.01$ vs. control.

most common findings were ectrodactyly, hemimelia, and adactyly (affecting 72.8%, 53.5%, and 8.8% fetuses per litter, respectively); the incidences of hemimelia and adactyly in the combination group were significantly ($p \leq 0.01$) higher than in the acetazolamide only group. All treatment regimens produced an increase in VSDs, although only the increase caused by the combination ibuprofen plus acetazolamide group was statistically significant ($p \leq 0.01$) (Table 5). Ibuprofen produced an increase in membranous VSD (3.7% fetuses per litter). Acetazolamide induced an increase in membranous VSD (5.9% fetuses per litter) and absent innominate artery (2.5% fetuses per litter; $p \leq 0.05$. The combination of acetazolamide and ibuprofen produced 41 fetuses with VSD (18.7% fetuses per litter; $p \leq 0.01$).

DISCUSSION

The concurrent inhibition of COX and carbonic anhydrase, achieved by coadministering ibuprofen and acetazolamide, did not produce DH (Table 5). Therefore, the difference in the profile of developmental anomalies produced by aspirin (VSD, DH, and MDs) (Gupta et al., 2003) and some NSAIDs (VSD only) (Cappon et al., 2003) may not be explained by ASA inhibiting carbonic anhydrase in addition to COX.

However, the teratogenic response after coadministration of ibuprofen and acetazolamide differed from what would have been anticipated if the effects were strictly additive (Tables 4 and 5). For example, ibuprofen alone produced 9 (3.7% per litter) fetuses with VSD, and acetazolamide treatment produced 15 (5.9% per litter) fetuses with VSD, but coadministration of ibuprofen and acetazolamide produced VSD in 41 (18.7% per litter) fetuses. In addition to these apparently synergistic effects, ibuprofen also potentiated the well-described acetazolamide-induced appendicular effects. Ibuprofen alone produced no fetuses with appendicular anomalies. Acetazolamide alone produced a range of appendicular anomalies with the most common being ectrodactyly (40.1% of fetuses per litter) and hemimelia (12.8% of fetuses per litter). After coadministration, increases in the incidence and severity of appendicular anomalies were observed. The incidences of the most common anomalies (ectrodactyly and hemimelia) increased to 72.8% and 53.5% of fetuses, respectively, in the combination ibuprofen and acetazolamide treated animals. Additionally, more severe expression of the appendicular effects was noted after coadministration. Acetazolamide alone produced 1 fetus with adactyly and 0 fetuses with polydactyly versus 20 and 3 fetuses, respectively, after coadministration of acetazolamide and ibuprofen.

Coadministration of acetazolamide and ibuprofen resulted in greater reductions in fetal weight (0.82 $\times$ control) than was caused by administration of either agent alone (0.95 $\times$ and 0.86 $\times$ control for ibuprofen and

acetazolamide, respectively) (Figure 1). However, there was no obvious difference between the distribution of fetal weights for normal fetuses as compared to the distribution of fetal weights for fetuses with VSD (Figure 2). The distribution of fetuses with appendicular anomalies may show a slight skewing toward fetuses with lower weights (Figure 3); although overall it does not appear that the increased magnitude of the reduced fetal weight in the combination group accounted for the increased incidence of developmental defects.

The influence of maternal toxicity on fetal development continues to be debated. In this study, coadministration of acetazolamide and ibuprofen produced greater maternal toxicity (as evaluated by measurements of body weight and feed consumption) than was caused by either agent alone (Tables 1 and 2). Maternal toxicity, demonstrated by reduced feed consumption and body weight gain, is sometimes associated with intrauterine growth retardation (IUGR), reduced fetal body weights, and delayed ossification, although maternal toxicity clearly does not always lead to fetal toxicity (Chahoud et al., 1999; Schardein, 1987). Maternal body weight gains from GD 9 to 12 were 10, 24, 24, and 39 g for the control, ibuprofen, acetazolamide, and combination groups, respectively. After GD 12, body weight gains were similar between groups. There is scant evidence to associate maternal toxicity with disruption of cardiovascular development such as VSD. Solomon and co-workers clearly demonstrated that disruption of maternal homeostasis by reducing maternal feed consumption, resulting in a 47% reduction in fetal weight, did not increase VSD incidence (Solomon et al., 1997). This is consistent with other work showing that impacting maternal homeostasis by limiting feed intake produces IUGR but not

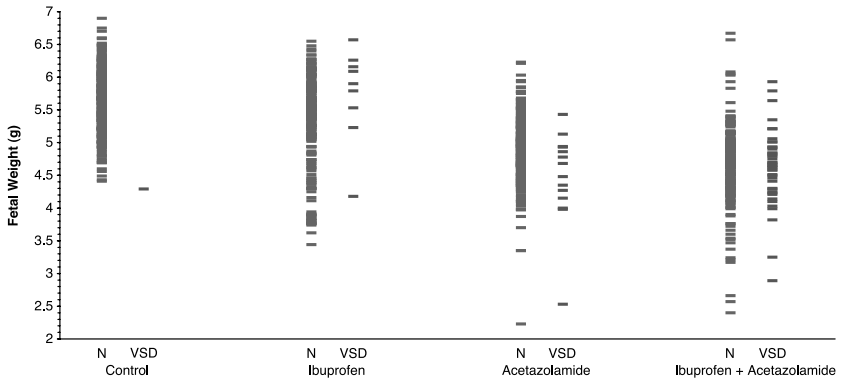

Figure 2: Distribution of normal fetuses and fetuses with VSD based on fetal weight; there were no obvious differences in the pattern of distribution between fetuses with or without VSD. N = fetuses without VSD (may have had other anomalies); VSD = fetuses with membranous VSD (findings described in Table 5).

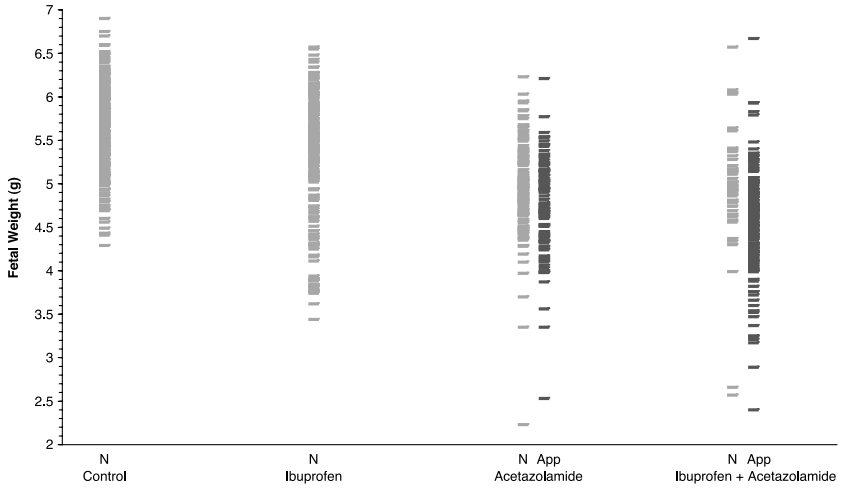

Figure 3: Distribution of normal fetuses and fetuses with appendicular anomalies based on fetal weight; there were no fetuses with appendicular anomalies in the control or ibuprofen only groups. There were no obvious differences in the pattern of distribution between fetuses with or without appendicular anomalies. N = fetuses without appendicular findings (may have had other anomalies); App = fetuses with appendicular anomalies (findings described in Table 4).

alterations in cardiovascular development (Ariyuki, 1987; De-Carvalho et al., 1994; Hannah and Moore, 1971). Maternal toxicity has been associated with deficits in skeletal development. For example, maternal toxicity in the form of chemically induced renal failure, during the sensitive window of heart development, increased minor skeletal anomalies but did not cause cardiovascular malformations (Kavlock et al., 1993). In a review of published studies, chemically induced maternal toxicity, demonstrated by reduced body weight gain or death, has been shown to be associated with a characteristic pattern of rib anomalies (Khera, 1985). However, the skeletal anomalies associated with maternal toxicity were relatively minor, in contrast to the severe appendicular anomalies (with obvious associated skeletal correlates) noted in this study. Therefore, although maternal toxicity may influence fetal development, the difference in magnitude of maternal toxicity produced by coadministration of ibuprofen and acetazolamide was not considered to be directly causal of potentiation of the developmental effects caused by that treatment.

Synergistic interactions between agents in the etiology of teratogenesis have been discussed for some time. Caffeine potentiates acetazolamide-induced limb malformations and also potentiates the teratogenic effects of hydroxyurea and 5-fluoro-2-deoxyuridine (DNA synthesis inhibitors), actinomycin D (RNA synthesis inhibitor), and cycloheximide and emetine (protein synthesis inhibitors) (Ritter et al., 1982). Potentiation of acetazolamide-induced

malformations has also been shown by coadministration of phenylephrine, serotonin, and ergotamine (Ugen and Scott, 1985, 1986, 1987).

Although the mechanism underlying potentiation of acetazolamide-induced fetal effects is not understood, one compelling hypothesis is that the potentiation is secondary to reduced uterine blood flow. Reduced uterine blood flow produced by physically clamping blood supply to the uterine horn potentiates the production of acetazolamide-induced anomalies (Ugen and Scott, 1987). Coadministration of the selective alpha-1-andrenergic receptor agonist phenylephrine potentiates acetazolamide-induced limb malformations; preantagonism with alpha-adrenergic antagonists (prazosin and/or phenoxybenzamine) prevents this potentiation (Ugen and Scott, 1987). The postulated mechanism is alteration in uterine blood flow. Alpha-adrenergic agonists reduced uterine blood flow, and carbonic anhydrase inhibition may indirectly reduce uterine blood flow, an effect that can be diminished by treatment with alpha-adrenergic antagonists (Ugen and Scott, 1986).

Maternal cardiovascular adaptations during pregnancy include decreases in uterine vascular resistance and a blunted pressor responsiveness to vasopressor substances (Bird et al., 2003; Gant et al., 1987). It has been suggested that prostaglandins, products of COX-mediated pathways, might play a role in maintaining the blood flow of the uterine circulation (Bird et al., 2003; Boura et al., 1994; Habermehl et al., 2000; Kimura et al., 1992; Kuhn and Stuart, 1987; Matsumoto et al., 1992), perhaps by mediating the reduced pressor responsiveness (Gant et al., 1987). Although there is little evidence to suggest that COX inhibition directly reduces uterine blood flow, treatment with the COX inhibitor indomethacin appears to restore the vascular responsiveness that is normally blunted during pregnancy (Gant et al., 1987). In this scenario, ibuprofen would reduce the pregnancy-induced blunted response to acetazolamide-induced reduction in uterine blood flow, in effect resulting in a greater reduction in blood flow for the combination ibuprofen plus acetazolamide group as compared to the acetazolamide group only.

Regardless of the mechanism for potentiation, the concurrent inhibition of COX and carbonic anhydrase by coadministration of ibuprofen and acetazolamide did not result in the production of DH. Therefore, the difference in the profile of developmental defects produced by some NSAIDs (VSD) and ASA (VSD, DH, and MD) cannot be explained by ASA inhibiting carbonic anhydrase and COX.

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