

**Original article:**

**SODIUM VALPROATE ENHANCES URETHANE TUMORIGENICITY  
IN LUNGS OF MALE BUT NOT FEMALE MICE**

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**ABSTRACT**

In the study, the possible effect of sodium valproate (NaVP) on urethane-induced lung tumors in mice has been evaluated. BALB/c mice (n = 120; 4–6 weeks old, both sexes) were used in the following groups: 1) urethane-treated, 2) urethane–NaVP-treated, 3) only NaVP-treated, 4) control. In the same groups, castrated male mice (n = 48) were investigated. Urethane was given by intraperitoneal injections 10 mg/mouse, twice a week, the total dose 50 mg/mouse. In NaVP-treated mice, the 0.4 % NaVP aqueous solution was offered to mice ad libitum. The duration of the experiment was 6 months. The number of tumors per mouse in urethane–NaVP-treated males was significantly higher than in those treated with urethane only ( $13.82 \pm 1.12$  vs  $6.77 \pm 0.43$ ,  $p < 0.0001$ ). No significant difference in the number of tumors per mouse was revealed while comparing the female urethane- and urethane–NaVP-treated groups ( $6.50 \pm 0.79$  vs  $8.15 \pm 0.55$ ,  $p = 0.105$ ). No difference in the number of tumors per mouse was found in urethane–NaVP-treated castrated males as compared with urethane-treated castrated males. However, in the urethane–NaVP-treated castrated males the number of tumors per mouse was significantly lower than in analogous non-castrated males ( $7.8 \pm 1.67$  vs  $13.82 \pm 1.12$ ,  $p < 0.01$ ). NaVP combined with urethane potentiates urethane tumorigenicity in BALB/c non-castrated but not in female and castrated male mice. These data indicate an important role of testosterone in the urethane–NaVP induced lung tumorigenesis.

**Keywords:** sodium valproate, urethane, mice, lung tumorigenesis, gender differences

**INTRODUCTION**

Urethane-induced lung tumors in mice models have been accepted for human lung adenocarcinoma investigations (Malkinson, 2001). BALB/c mice are considered as a medium susceptible for the development of lung tumor by urethane and can be modified

by the potential modulatory agents (Malkinson and Beer, 1983; Uleckiene et al., 2005). There are no studies on the effect of sodium valproate (NaVP) on urethane-induced lung tumors in mice. The aim of our study was to investigate the effect of NaVP on lung tumorigenesis induced by urethane,

a chemical lung carcinogen, in BALB/c mice of both genders.

NaVP is a medicine preparation which currently is used for the treatment of a wide variety of neurological disorders (Evers, 2008; Bowden, 2009). NaVP increases the turnover of gamma-aminobutyric acid (GABA) and thereby potentiates GABAergic functions (Loscher, 2002). GABA may affect cancer growth by activating GABA receptors. The gene expression investigation of GABA receptors in tissues of non-small cell lung cancers showed the gene expression of GABA receptor phenotypes to be correlated with cancerogenesis and clinical prognosis (Zhang et al., 2013). Similarly, GABA stimulates human hepatocellular carcinoma growth through the overexpressed GABA A receptor (Li et al., 2012).

The GABA A receptor is an ionotropic receptor. Its subunits form a functional chloride channel (Fritschy and Mohler, 1995; Bureau et al., 1997). The GABA A receptor is rapidly activated by NaVP in the brain cells (Armijo et al., 2000). The GABA A receptor subunits are expressed in Wistar rat kidney proximal convoluted and straight tubules and in other tissues (Sarang et al., 2001; Li et al., 2012; Zhang et al., 2013). NaVP has shown to enhance the urinary excretion of sodium and chloride ions in Wistar rats of both genders, but the 24-hour chloriduretic response was found to be gender-related (Grikinienė et al., 2005). Chloride is recognized to have an important role in tumor genesis: the intracellular chloride concentration would be one of the critical messengers in cell growth/proliferation and differentiation processes (Shiozaki et al., 2006; Hiraoka et al., 2010; Ohsawa et al., 2010).

Furthermore, NaVP defines a novel class of HDAC inhibitors that induce differentiation of transformed cells and show antitumor properties in clinical and preclinical studies (Göttlicher et al., 2001; Kawagoe, 2002; Santoro et al., 2013). In chronic rat and mouse studies using NaVP, an increased incidence of subcutaneous fibrosarcoma occurred in male rats at the higher dosage level,

and a dose-related trend for an increased incidence of benign pulmonary adenomas was observed in male mice (McEvoy, 1991). Many aspects of the contribution of the NaVP pharmacological mechanisms and their significance in gender-related tumorigenesis have not been investigated.

Our data have shown that NaVP together with urethane increases urethane tumorigenicity in male but not in female mice. In contrast, in the groups of castrated males, no synergistic NaVP and urethane effect on lung tumor numbers was found. The possible mechanisms of the NaVP gender-related effect are discussed in the article.

## MATERIALS AND METHODS

In the study, we used the BALB/c mouse lung adenoma assay which is one of the assays often used for investigating the possible cancer modulative agents. The examined BALB/c mice are characterized by a high sensitivity to the pulmonotropic carcinogen urethane. BALB/c mice (n = 120; 4–6 weeks old, both sexes) and castrated BALB/c male mice of similar age (n = 48) were used in the study. The animals were purchased from the Animal Facility of the Lithuanian University of Health Sciences, Veterinary Academy (Kaunas, Lithuania). The experiments were performed in compliance with the relevant laws and institutional guidelines. Permissions of the State Food and Veterinary Service of Lithuania to use experimental animals for research were obtained (No. 0177/30/04/2008/2 & 25/07/2013). Male castration was performed by removing testes at the age of 5–6 weeks. The animals were housed in standard colony cages with free access to food and acclimatized for one week before the study; they were housed in the conditions of constant temperature ( $21 \pm 1$  °C), humidity, and the light/dark cycle (12 h/ 12 h). A commercial pellet diet and fresh drinking water were provided ad libitum.

NaVP was from Sigma-Aldrich Chemie GmbH, Germany, and urethane (ethyl carbamate) was from Fluka.

Intact male and female mice were randomly divided into eight groups – four groups for each gender ( $n = 15$  in a group):

- 1) treated with urethane,
- 2) treated with urethane plus NaVP,
- 3) treated with NaVP only,
- 4) intact control.

In the same four groups ( $n = 15$  per group), castrated male mice were investigated. In the urethane-treated groups, 10 mg urethane/mouse in 0.2 ml sterile physiological solution was given twice a week by intraperitoneal injections (5 doses, total dose 50 mg/mouse). The second and third groups of both genders were treated per os with NaVP 0.4 % aqueous solution (in the second group starting two weeks prior to urethane administration) for six months. The NaVP solution was offered to the animals ad libitum (in aluminum foil-wrapped bottles to avoid light-induced decomposition). The control and urethane groups were given water which was their only source of drinking. After six months, animals that survived were killed in a CO<sub>2</sub> camera. Lungs were fixed in a 10 % buffered formalin solution. Randomly selected tumors were taken for histological evaluation. Histological examination was performed in each case when observable lung pathology (suspected not identified or malignant tumor) was present. The percentage of animals with lung tumors, the mean number of tumors per tumor-bearing mouse, tumor growth and histological type were considered in both genders only in mice that survived up to the end of the experiment. The results were statistically processed using Student's *t*-test. Data were expressed as a mean  $\pm$  SEM. The difference at the value of  $p < 0.05$  was considered significant.

## RESULTS

### *Mice survival in the study groups*

*Non-castrated mice.* Mice of both genders in control and NaVP-treated groups survived until the end of the experiment. In the male urethane-treated group, from 15 mice 13 survived: one mouse died in the 4<sup>th</sup> and the other in the 5<sup>th</sup> month of the experiment.

In the urethane–NaVP male group, 11 mice (three mice died in the 1<sup>st</sup>–3<sup>rd</sup> months and one in 5<sup>th</sup> month) survived. In the female urethane-treated group, five mice did not survive: three mice died in the 1<sup>st</sup>–3<sup>rd</sup> months, one in the 4<sup>th</sup> and one in the 5<sup>th</sup> month of the experiment. In the urethane–NaVP-treated group, two mice perished (one in the 1<sup>st</sup> and the other in the 5<sup>th</sup> month). This implies that young animals are sensitive to urethane and urethane–NaVP toxicity. Autopsies of mice that perished during the 5<sup>th</sup> month revealed that in urethane- and urethane–NaVP-treated groups benign lung tumors had developed in all cases, but data on these animals were not taken for statistical assessment.

*Castrated male mice.* Among castrated mice, all mice survived in the following groups: in the castrated control as well as in NaVP-treated and in urethane-treated groups. In the male urethane–NaVP-treated group, nine mice survived up to six months of the experiment (two mice died in the 2<sup>nd</sup>–3<sup>rd</sup> and four in the 3<sup>rd</sup>–4<sup>th</sup> months). The prevalence of perished mice in the urethane–NaVP-treated group is indicative of the toxicity of this combination for castrated mice.

### *Gender-related differences in the number of lung tumors per mouse in non-castrated mice groups*

No mice with lung tumors were found in control animals of both genders and in NaVP-treated female groups. Four small ( $< 1$  mm) tumors were found in the lungs of two mice in the NaVP-treated male group (one in one mouse and three in the other).

All urethane-treated mice of both genders developed tumors. No significant difference in the number of tumors (lung tumors per mouse) was detected when comparing urethane-treated male and female mice groups (Table 1).

Urethane-caused adenomas may be found in any lobe of a lung and are often situated just beneath the pleura, and are recognized by their nodular, pearly, grey-white appearance contrasting with the more pink

color of the normal lung parenchyma (Figure 1).

When comparing the number of tumors per mouse in male mice treated with urethane and those treated with urethane plus NaVP, lung tumors were found statistically significantly more often in males treated with urethane in combination with NaVP ( $6.77 \pm 0.43$  vs  $13.82 \pm 1.12$ ,  $p < 0.0001$ ; Table 1). The number of tumors per male mouse treated with urethane ( $n = 13$ ) according to tumor diameter was per subgroup as follows:  $3.85 \pm 0.32$  in the subgroup with the tumor diameter  $\leq 1.0$  mm,  $2.31 \pm 0.45$  in 1.0 to 2.0 mm and  $0.23 \pm 0.17$  in the  $> 2.0$  mm subgroup. In male urethane–NaVP-treated mice ( $n = 11$ ), the number of tumors per mouse was as follows: with the diameter  $\leq 1.0$  mm  $8.09 \pm 0.94$ , with the diameter 1.0 to 2.0 mm  $5.45 \pm 0.72$ , and with  $> 2.0$  mm  $0.27 \pm 0.14$ . NaVP in mice treated with urethane caused an increase in the number of tumors per mouse in subgroups with the tumor diameter  $\leq 1.0$  mm ( $p < 0.001$ ) and 1.0 to 2.0 mm ( $p < 0.001$ ). This fact indicates that NaVP causes the formation of new tu-

mors and increases the progression of tumors in male BALB/c mice (Figure 2).

No statistically significant difference was found when comparing the number of tumors per mouse in females treated with urethane and those treated with the urethane and NaVP combination ( $6.50 \pm 0.79$  vs  $8.15 \pm 0.55$ ,  $p = 0.105$ ; Table 1). The number of tumors per female mouse treated with urethane ( $n = 10$ ) according to tumor diameter was as follows:  $4.20 \pm 0.59$  with tumor diameter  $\leq 1$  mm,  $1.90 \pm 0.43$  with the diameter 1.0 to 2.0 mm, and  $0.4 \pm 0.31$  with the diameter  $> 2.0$  mm. In the female urethane–NaVP-treated group ( $n = 13$ ), the number of tumors per mouse was as follows:  $5.0 \pm 0.46$  with the diameter  $\leq 1$  mm,  $3.08 \pm 0.51$  with the diameter 1.0 to 2.0 mm, and  $0.08 \pm 0.08$  with the diameter  $> 2.0$  mm (Figure 2).

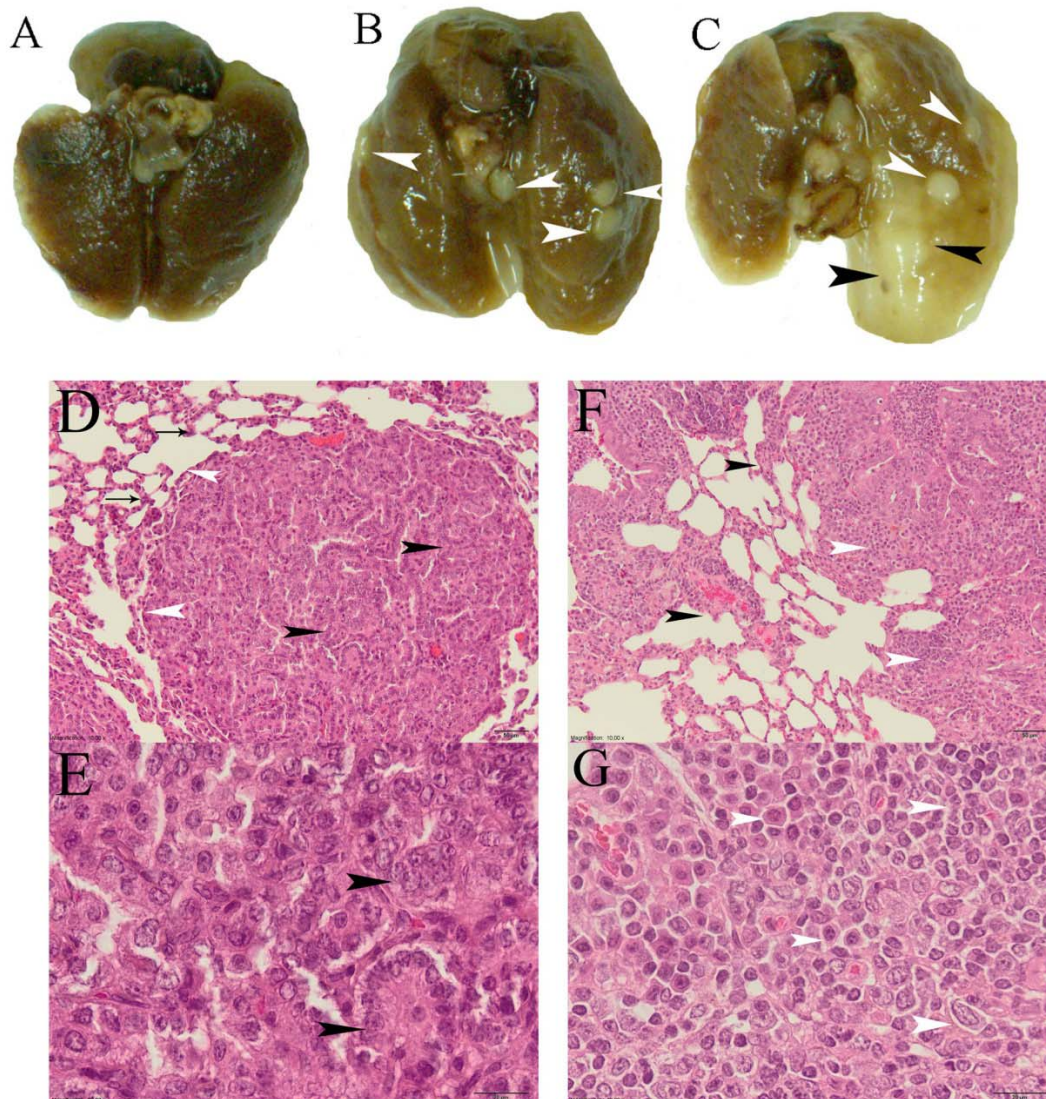
No significant difference in the number of tumors per mouse was revealed while comparing the female urethane- and urethane–NaVP-treated groups, both in general and in separate subgroups.

**Table 1:** Incidence of lung tumors in BALB/c mice of the study groups

Groups	Initial data		Data after 6 months			
	<i>n</i>	body weight (g) <i>m</i> ± <i>SDEV</i>	<i>n</i>	body weight (g) <i>m</i> ± <i>SDEV</i>	mice with tumors (%)	tumors in mouse <i>m</i> ± <i>SEM</i>
<b>Intact control:</b>						
male	15	14.3 ± 2.3	15	29.5 ± 1.9	0	0
female	15	12.0 ± 1.5	15	24.3 ± 1.1	0	0
<b>Urethane-treated:</b>						
male	15	14.9 ± 1.7	13	27.8 ± 2.7	100	6.77 ± 0.43
female	15	13.2 ± 1.1	10	21.4 ± 2.9	100	6.50 ± 0.79
<b>Urethane–NaVP-treated:</b>						
male	15	15.9 ± 2.3	11	26.6 ± 2.9	100	13.82 ± 1.12*
female	15	13.6 ± 1.3	13	20.4 ± 3.7	100	8.15 ± 0.55**
<b>NaVP-treated:</b>						
male	15	12.5 ± 1.2	15	27.7 ± 2.6	13.3	0.19 ± 0.14
female	15	11.4 ± 1.3	15	20.0 ± 3.2	0	0

\*  $p < 0.0001$  in comparison with the male group given urethane only.

\*\*  $p < 0.01$  in comparison with the male group given urethane–NaVP.



**Figure 1:** Lung specimens. **A** – control; **B** – urethane-induced lung multiple benign adenomas; **C** – lungs with advanced tumor (black arrowheads) – adenomas after urethane–NaVP treatment (white arrowheads); **D** – histological slide of lungs with benign adenoma. White arrowheads show well established borders of the tumor, black arrowheads – tumor cells with rounded regular nuclei, and arrows show alveolar/bronchial hyperplasia (bar 50  $\mu$ m); **E** – adenoma, higher magnification. Tumor cells have regular oval nuclei, in some places cells have papillary arrangement (bar 20  $\mu$ m); **F** – pulmonary adenocarcinoma. Black arrowheads show irregular borders of the tumor and invasive growth, white arrowheads – pleomorphic tumor cells (bar 50  $\mu$ m); **G** – malignant tumor, higher magnification. Cells have pleomorphic nuclei, an increased number of nucleoli, some cells have very dark nuclei (bar 20  $\mu$ m).

A comparison of tumor numbers in urethane–NaVP-treated males and analogous data of the female group has shown a significant difference in the following subgroups: the number of tumors was higher in males with the tumor diameter  $\leq 1$  mm ( $8.09 \pm 0.94$  vs  $5.0 \pm 0.46$ ,  $p < 0.01$ ), in the subgroup with

the diameter 1.0 to 2.0 mm ( $5.45 \pm 0.72$  vs  $3.08 \pm 0.51$ ,  $p < 0.01$ ), and in the subgroup with the diameter  $> 2.0$  mm ( $0.27 \pm 0.14$  vs  $0.08 \pm 0.08$ ,  $p < 0.01$ ). These data show that the NaVP effect on lung tumorigenesis is gender-related.



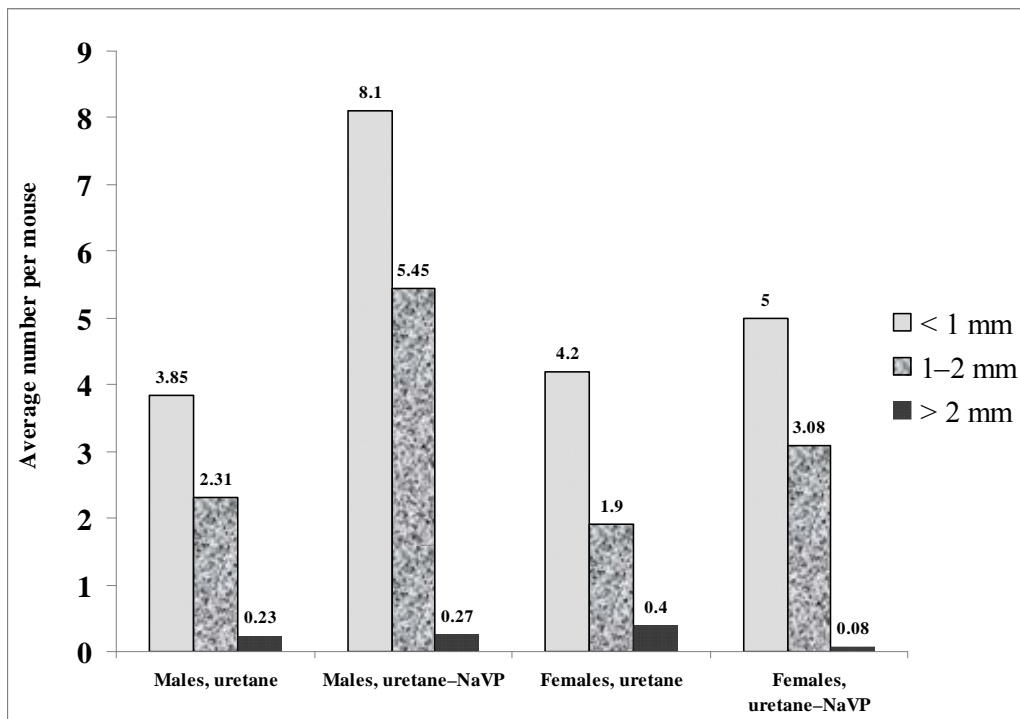
**The number of tumors per mouse in castrated male groups**

Three spontaneous tumors were found in the lungs of two control mice (two tumors in one and one more in another mouse). Three mice with lung tumors were found in the NaVP-treated castrated male group. All urethane-treated and urethane–NaVP-treated mice developed tumors. No statistically significant difference was found when

comparing the number of tumors per mouse in castrated male mice treated with urethane and in those treated with the urethane and NaVP combination. The number of tumors per castrated animals treated with urethane in subgroups according to tumor diameter showed no statistically significant difference as compared with data on the urethane–NaVP-treated group (Table 2).

**Table 2:** Incidence of lung tumors in castrated BALB/c male mice of the study groups

Groups	n	body weight (m ± SD)	mice with tumor (%)	Data after 6 month			
				all	<1.0 (mm)	1.0–2.0 (mm)	>2.0 (mm)
Castrated control	15	23.4 ± 2.4	13.3	0.2 ± 0.15	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
Urethane-treated	15	22.7 ± 3.6	100	8.0 ± 1.21	4.2 ± 0.7	3.8 ± 0.7	0.5 ± 0.2
Urethane–NaVP-treated	9	24.4 ± 4.6	100	7.8 ± 1.67	3.9 ± 1.7	3.4 ± 0.4	0.5 ± 0.6
NaVP-treated	15	24.4 ± 2.5	6.7	0.13 ± 0.1	0	0.2 ± 0.2	0



**Figure 2:** Incidence of benign lung tumors in BALB/c mice of both genders in urethane- and in urethane–NaVP-treated groups according to data on subgroups by tumor size (diameter ≤ 1.0 mm, 1.0 to 2.0 mm and ≥ 2.0 mm).

No significant difference in the number of tumors per mouse was detected when comparing intact male control and castrated male control groups as well as non-castrated and castrated urethane-treated male groups (Tables 1 and 2;  $p > 0.05$ ). When comparing the number of tumors per mouse in castrated male mice urethane–NaVP-treated and non-castrated urethane–NaVP-treated groups, lung tumors were found statistically significantly more often in non-castrated males ( $7.8 \pm 1.67$  vs  $13.82 \pm 1.12$ ,  $p < 0.01$ ; Tables 1 and 2).

### **Cases of malignant tumors**

A randomly selected histological examination performed in each case of observable lung pathology when not identified or malignant tumors were suspected revealed several cases of lung tumor adenocarcinoma in non-castrated urethane- and urethane–NaVP-treated mice groups of both genders. Cases of adenocarcinoma (Figure 1) were identified by a histological examination of lungs and found to occur together with benign tumors.

In castrated male mice, a few malignant tumors were found in NaVP-treated, urethane-treated, and in urethane–NaVP-treated mice. Adenocarcinoma was revealed in cases when the tumor diameter was more than 1.5 mm (results of a detailed histological examination of adenocarcinoma cases will be published separately upon analysing all tumors in all mice groups).

## **DISCUSSION**

Urethane is a chemical carcinogen present in tobacco leaves, tobacco smoke (Schmetz et al., 1978), formed by the reaction of urea with alcohol (Wang et al., 2007). Urethane specifically initiates the development of lung tumors from airway epithelial cells in mice. The tumorigenic effect of urethane directly depends on its dose (Malkinson, 2001). BALB/c mice are considered susceptible to the development of lung tumor by urethane, and lung tumors can be modified by the influence of different modulatory

agents (Malkinson and Beer, 1983; Uleckiene et al., 2005).

In our study model, all urethane-treated mice of both genders developed lung tumors. No gender-related difference in the number of mice with lung tumors as well as in tumors per mouse was detected between urethane-treated male and female mice. The study data show that the number of pulmonary tumors per mouse in the urethane-treated males and in males treated with urethane plus NaVP, lung tumors were observed statistically significantly more often in the non-castrated male group treated with urethane in combination with NaVP. No significant difference was found between analogous female mice groups. No lung tumors were found in untreated intact control groups of both genders. Of all male mice in the NaVP-treated group, 13.3 % developed benign lung tumors. No mice with lung tumors were found in the NaVP-treated female group.

The study data show that NaVP in the urethane-treated BALB/c mice could act synergistically with urethane as an oncogenic factor in male but not in female mice. Sex differences in mouse models of lung cancers were reported (Festing et al., 1994). The importance for lung oncogenesis in both spontaneous and carcinogen-induced pulmonary cancers and spontaneous pulmonary adenomas in mice is determined by multiple genetic loci that differ between female and male mice (Berndt et al., 2011). The study data show that NaVP in the urethane-treated castrated BALB/c male mice did not act synergistically with urethane as an oncogenic factor in male, and this fact indicates that testosterone plays an important role in the tumorigenic process in this model. There are no studies on NaVP influence on urethane-related lung tumorigenesis in mice.

Chronic NaVP toxicity studies showed that orally administered NaVP to Sprague Dawley rats and ICR (HA/ICR) mice at doses of 80 and 170 mg/kg/day for two years developed an increased incidence of subcutaneous fibrosarcoma in male rats at a

higher NaVP dosage level, and a dose-related trend for an increased incidence of benign pulmonary adenomas was observed in male mice (McEvoy, 1991).

NaVP increases the GABA turnover and potentiates GABAergic functions, reducing the activity of the GABA degrading enzymes (Godin et al., 1969; Loscher, 2002). NaVP may enhance neuronal responsiveness to endogenous GABA. The effect of NaVP on cellular mechanisms may be sex-specific with the different response on basal GABA levels in males and females (Dodge et al., 2000). The specificity of NaVP for GABA suggests that this interaction may be an important mechanism through which NaVP exerts its pharmacological effects. The GABA A receptor is rapidly activated by NaVP in brain cells (Armijo et al., 2000). The GABAergic neuronal activity was about two-fold greater in male than female rats (Grattan and Selmanoff, 1997). A comparison between diestrous female rats and chronically castrated animals indicated that endogenous GABA release in ovariectomized rats was only 60–70 % of that in diestrous animals, while the basal GABA levels are also significantly reduced in estrogen-treated ovariectomized rats (Ondo et al., 1982). In male rats, castration decreases the activity of GABAergic neurons, suggesting that GABAergic neurons are tonically stimulated by testosterone. Testosterone replacement prevents the castration-induced decrease in GABA (Grattan and Selmanoff, 1993).

GABA A receptors are also found outside synapses and on non-neural cells, cancerous cells included. GABA A receptor  $\theta$  subunit (GABRQ) forms a functional ionotropic chloride channel (Fritschy et al., 1995; Bureau et al., 1997). Such extrasynaptic receptors have a high affinity for GABA and open the chloride channels at low ambient GABA concentrations (1 mM). This leads to changes in the membrane potential (Belelli et al., 2009). Opening the GABA A receptor chloride channels leads to a chloride efflux and depolarisation of the membrane (Zhao et al., 2011). The plasma cell membrane poten-

tial influences cell proliferation (Morokuma et al., 2008; Sundelacruz et al., 2009). Neurotransmitters that change the membrane potential contribute to the control of cell proliferation (Martins and Pearson, 2008). GABA has been shown to regulate the proliferation of several cell types, including stem cells (Andang et al., 2008), cortical progenitor cells (Nguyen et al., 2003), immune cells (Bjurstom et al., 2008; Mendu et al., 2011), mouse chondrogenic ATDC5 cells (Tamayama et al., 2005), modulate the proliferation, migration and differentiation of neuronal cells (Neelands et al., 1999; Haydar et al., 2000; Meier et al., 2003).

The gene expression investigation of GABA receptors in tissues of non-small cell lung cancers and non-cancerous tissues showed that the gene expression of GABA receptor phenotypes was correlated with tumorigenesis and clinical prognosis (Zhang et al., 2013). The GABRQ is overexpressed in hepatocellular carcinoma cells, but not in a normal cell line, and GABA in the liver promotes the proliferation of cancer cells through GABRQ (Li et al., 2012). GABA could inhibit colon cancer migration associated with the norepinephrine-induced pathway (Joseph et al., 2002). The effects of GABA and GABA receptors show that GABA-associated pathways could act positively or negatively in regulating cancer cell behavior. The injection of a combination of oestrogen and progesterone produced a greater reduction in GABA A receptor binding in mouse forebrain membranes, indicating that gonadal steroids contribute to the modulation of GABA A receptor binding in the cells of male and female mice (Akinci and Johnston, 1997).

There could be other factors that may contribute to NaVP gender-related induction of cell proliferation and tumorigenesis responses, for example, the link of GABA mechanisms with the ion cotransporter activity expression regulating the intracellular chloride concentration. The depolarizing effects of GABA are promoted by the relative accumulation of chloride inside the cells,



leading to chloride efflux once GABA A receptor channels open. Intracellular chloride regulation and the control of GABA A receptor signaling are effected through K-Cl co-transporter (Delpire and Mount, 2002). To counteract their effects, the K-Cl cotransporter exports these ions thus decreasing intracellular chloride (Galanopoulou, 2008a). In male rat neurons, the K-Cl cotransporter is less expressed than in female (Galanopoulou, 2008b). The possible mechanisms of K-Cl cotransporter activity was demonstrated in the modulation of tumor development and progression (Shen et al., 2004).

NaVP has been shown to enhance the urinary excretion of chloride in Wistar rats of both genders, but the 24-hour chloriduretic response in male was significantly higher than in female rats (Grikinienė et al., 2005). GABA A receptor subunits are expressed in Wistar rat kidney proximal convoluted and straight tubules and in other tissues (Sarang et al., 2001; Li et al., 2012; Zhang et al., 2013). Chloride is recognized to have an important role in tumor genesis: the intracellular chloride concentration would be one of the critical messengers in cell growth/proliferation and differentiation processes (Shiozaki et al., 2006; Hiraoka et al., 2010; Ohsawa et al., 2010).

NaVP is recognized as a novel class of histone deacetylases (HDAC) inhibitors that induce the differentiation of transformed cells and show antitumor properties in clinical and preclinical studies, both in vitro and in vivo, by modulating multiple pathways including cell cycle arrest, cell differentiation, apoptosis (Göttlicher et al., 2001; Kawagoe et al., 2002). Researchers have summarized preclinical and clinical data on positive NaVP effects in different types of tumors (Blaheta and Cinatl, 2002; Duenas-Gonzalez et al., 2008). Recent data indicate that HDAC play a dual role in tumorigenesis: oncosuppressive in the early stages, and oncogenic in established tumor cells in mice models (Santoro et al., 2013). Our study results show the opposite – negative – effect of NaVP on the progression of ure-

thane-induced lung tumors in the mice model. We have found no other gender-related mice studies on NaVP effects on tumorigenesis in the literature. Furthermore, there are data to show that NaVP increases the level of histone H3 acetylation in the brain with a gender-related effect: multiple NaVP selectivity improved the early olfactory learning experience in male but not in female 8-day-old mice pups (Burenkova et al., 2013).

It is possible to hypothesize that NaVP, by changing GABA A receptor activity, the intracellular chloride level, and possibly modulating HDAC, plays an important gender-related modulative role in urethane-induced lung tumorigenesis in mice.

In our study, there were cases of malignant lung tumor in urethane- and urethane–NaVP-treated mice groups. The effect of urethane is known to be related with benign adenoma or adenocarcinoma lung tumorigenesis (Malkinson, 2001; Regala et al., 2011). The data of our study show that NaVP may influence adenocarcinoma frequency in urethane-caused lung tumor genesis. This effect was observed in tumors with the diameter more than 1.5 mm (a detailed analysis of adenocarcinoma cases with the description of histological examination results will be published separately).

Data of the present study show that NaVP, used together with urethane, synergistically enhances urethane tumorigenicity in lungs of only non-castrated male BALB/c mice, but not in castrated ones. To check our findings on NaVP induction and promotion of gender-related lung tumorigenesis in the urethane mice model, the further studies on the importance of the NaVP gender-related action on pharmacological and pathophysiological mechanisms are necessary.

#### ACKNOWLEDGMENTS

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## CONFLICT OF INTERESTS

The authors have no conflict of interests.

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