Original Article

Oral administration of the anti-proliferative substance taurolidine has no impact on dextran sulfate sodium-induced colitis-associated carcinogenesis in mice

Ansgar Michael Chromik**, Sebastian Huss#, Hayssam Osseili, Adrien Daigeler², Sabine Kersting, Dominique Sülberg, Ulrich Mittelkötter, Thomas Herdegen³, Waldemar Uhl, Annette M. Müller¹

Departments of General and Visceral Surgery, St. Josef Hospital, ¹Plastic Surgery, University Hospital Bergmannsheil, Ruhr-University Bochum, ¹Department of Pediatric Pathology, University of Bonn, ¹Institute of Pharmacology, University Hospital of Schleswig-Holstein, Campus Kiel, Germany, #both authors contributed equally to this publication

E-mail: a.chromik@klinikum-bochum.de
*Corresponding author

Published: 16 April, 2010 DOI: 10.4103/1477-3163.62536
Received: 11 January, 2010
Accepted: 14 March, 2010

This article is available from: http://www.carcinogenesis.com/content/9/1/5
© 2010 Chromik.

Abstract

**Background:** New chemopreventive strategies for ulcerative colitis (UC)-associated dysplasia and cancer have to be evaluated. Taurolidine (TRD) has anti-inflammatory, anti-proliferative and anti-neoplastic properties with almost absent toxicity. The aim of the study was to determine whether TRD decreases dysplasia in the well-characterized Dextran Sulfate Sodium – Azoxymethane (DSS-AOM) animal model for UC-associated carcinogenesis. **Material and Methods:** The DSS-AOM model of carcinogenesis was induced in female inbred C57BL/6 mice. Half of the mice were treated with TRD, the other served as control. After 100 days macroscopic, histological and immunohistochemical (β-Catenin, E-Cadherin, SOX9, Ki-67, Cyclin-D1) examination of the colon was performed. **Results:** Incidence, multiplicity, grading and growth pattern of adenomas did not differ significantly between TRD and control group. In all animals, inflammatory changes were absent. Immunohistochemistry revealed increased expression of Ki-67, β-catenin, SOX9 and Cyclin-D1 in adenomas compared to normal mucosa – without significant difference between TRD and control treatment. **Conclusion:** Oral administration of TRD has no impact on DSS-induced colitis-associated carcinogenesis. However, SOX9 and Cyclin-D1 representing key members of the Wnt pathway have not yet been described in the DSS-AOM model of carcinogenesis – underlining the importance of this oncogenic pathway in this setting.

**Keywords:** Carcinogenesis, C57BL6 mice, Dextran Sulfate Sodium, experimental colitis, inflammatory bowel disease, Taurolidin, Taurolin, TRD

INTRODUCTION

**Ulcerative colitis-associated colorectal cancer**

Patients with ulcerative colitis (UC) have a significantly increased risk of developing colorectal dysplasia and colorectal cancer (CRC) during their lifetime.[1] Although ulcerative colitis-related colorectal cancer accounts for only 1-2% of all CRC cases in the general population, it is responsible for 10-15% of all deaths in UC patients.[2] For the individual UC patient, risk of malignancy is continuously increasing with duration of the disease and therefore several surveillance strategies have been developed.[3] However, in UC patients with a disease history of 30 or more years, the cumulative risk for CRC ranges in recent studies between 10-18%, depending on screening methods, geographic and other epidemiologic factors.[4,5]
Chemoprevention

Despite surveillance colonoscopy or prophylactic colectomy, the concept of chemoprevention has gained increasing importance during the last years. Many pharmacological agents have been evaluated for their chemopreventive features for UC-associated CRC. The ideal chemopreventive agent would be effective at preventing neoplastic progression, safe (i.e. without side-effects) and inexpensive. The most frequently used chemopreventive agents in UC patients are 5-aminosalicylic acid (5-ASA) compounds like mesalazine or sulfasalazine as well as ursodeoxycholic acid (UDCA), which is applied in patients with primary sclerosing cholangitis (PSC). Whereas the chemopreventive role for UDCA in PSC patients is broadly accepted, there is still ongoing debate about the chemopreventive capacity of 5-ASA derives in UC patients without PSC. Although there are numerous studies supporting the chemopreventive efficacy of 5-ASA, other authors could not show significant reduction in UC-related CRC or dysplasia. As a result, further therapeutic targets and corresponding pharmacological candidates have to be evaluated for chemoprevention in UC-related CRC.

Taurolidine

Taurolidine (TRD) – a derivate of the aminosulfonic acid Taurin – has anti-inflammatory, anti-proliferative and anti-neoplastic properties. So far, TRD was mainly used in the treatment of peritonitis and catheter-related bloodstream infections due to its capacity to inactivate bacterial cell wall components, e.g. lipopolysaccharides and various exotoxins. The anti-proliferative and anti-neoplastic effects of TRD have been investigated in several tumor cell lines in vitro as well as in vivo. Inhibition of proliferation and cell death induction by TRD seems to be a multifaceted process and remains to be fully elucidated. However, cell death-inducing mechanisms of TRD include the mitochondrial cytochrome-c-dependent apoptotic pathway, inhibition of protein synthesis as well as activation of autophagy. Application of TRD in patients with advanced gastric cancer and glioblastoma showed promising clinical results with almost absent toxicity and further oncological trials with iv application of TRD are currently being conducted.

DSS-AOM model of carcinogenesis

The Dextran Sulfate Sodium – Azoxymethane (DSS-AOM) model is a well-characterized experimental model for UC-associated CRC. Mice that are exposed to a single injection of the classic colon carcinogen AOM prior to cyclic administration of DSS in drinking water will develop chronic inflammatory changes as well as dysplasia and carcinoma with pathological features that resemble those of human UC-associated neoplasia. The extent of neoplastic lesions depends on several factors like strain susceptibility as well as duration, dosage and schedule of cyclic DSS application.

Taurolidine as chemopreventive agent?

Besides our experience with TRD as apoptosis-inducing and anti-proliferative agent in colorectal and other cancer cell lines in vitro, we could recently report that oral application of TRD is well tolerated and significantly ameliorates chronic DSS colitis in C57/B6 mice. However, the potential role of TRD in chemoprevention of colitis-associated CRC has not been investigated so far. The aim of this study was therefore to evaluate the chemopreventive capacity of oral TRD in the murine DSS-AOM model of carcinogenesis.

MATERIALS AND METHODS

DSS-AOM model of carcinogenesis

The DSS-AOM model of carcinogenesis was induced in female inbred C57BL/6 mice (Charles River, Sulzfeld, Germany, body weight 22–25g) which were randomized into two groups with n = 9 animals each. On Day 0, all animals received a single intraperitoneal injection of Azoxymethane (AOM) (Sigma-Aldrich, Munich, Germany) at a dose level of 10 mg/kg body weight and received demineralized water for seven days. Thereafter, a Dextrane sodium sulfate (DSS) colitis (molecular weight 36–44 kDa, MP Biomedicals, Aurora, OH, USA) was induced by a three-cyclic administration of 0.75% DSS solution in demineralized water for five days, followed by a five-day DSS-free interval. During the DSS-free interval and after finishing the third DSS cycle, one group received 0.2% TRD (w/v) in demineralized water (TRD group, n = 9), which has been shown to exert anti-inflammatory effects in previous studies. As control, a second group received demineralized drinking water only (control group, n = 9). After 100 days all animals were killed under deep anesthesia. Mice were housed three per cage with diet and fluids ad libitum. During the first 36 days (i.e. induction of DSS colitis), disease activity was quantified every day using the Disease activity index described elsewhere.

Histological evaluation of H and E sections

After sacrificing the mice after 100 days, the colon was removed and rinsed with normal saline. The length of the colon was measured and macroscopic photographs were taken. Thereafter, the colon was divided into four parts, i.e. cecum, proximal, mid and distal colon and fixed in 10% buffered formaline for 24 h. Histological examination of the entire colon was performed on paraffin-embedded sections after H and E staining. Histological slides were evaluated independently by two investigators (S.H. and A.M.M.) blinded to the respective groups. Proliferative colonic...
mucosal changes e.g. hyperplasia, aberrant crypt foci (ACF), gastrointestinal intraepithelial neoplasia (GIN), adenoma and adenocarcinoma were diagnosed according to the Pathology of Mouse Models of Intestinal Cancer: Consensus Report and Recommendations.\textsuperscript{[30]} With respect to inter-observer agreement, differences were found in less than 5% of observations. Any differences in grading were resolved by joint examination.

**Immunohistochemistry**

Immunohistochemistry (IHC) for $\beta$-catenin, E-Cadherin, SOX9, Ki-67, and Cyclin-D1 was performed on 4-µm-thick paraffin-embedded sections by use of the peroxidase-conjugated avidin-biotin method. Deparaffinized and rehydrated sections were incubated with the following primary antibodies: anti-$\beta$-catenin (1:1000, Transduction Lab., Lexington, UK), anti-E-Cadherin (1:50, Santa Cruz, Europe), anti-Ki-67 (1:25, DAKO, Germany), anti-Cyclin-D1 (1:25, DCS, Hamburg, Germany) and anti-SOX9 (1: 500, RnD Systems, Germany). Immunoreactions were visualized by using 3-amino-9-ethylcarbazole (AEC) as a substrate (DAKO Real Detection System, Ref: K5003, Germany) following incubation with biotinylated secondary antibodies. Based on the immunoreactivity score (IRS) by Remmele a scoring system was used to describe the staining intensity (negative, weak, moderate, and strong) and proportion (0-100%) of cells stained.\textsuperscript{[41]} Integer values were assigned to intensity scores (0-3) and proportion of stained cells and multiplied to provide a single integrated score for each staining. The data were reduced to an ordinal scale of 0 to 3. For $\beta$-catenin three different cell localizations, nuclear (N), cytoplasmic (C) and membrane (M) were analyzed. For the estimation of nuclear staining by Cyclin-D1 and Ki-67, the percentage of stained nuclei were scored and integer values were assigned to the proportion of cells stained (0-4).\textsuperscript{[42]} All histological quantifications were first performed as independent analysis by two observers (S.H and A.M.M.) as described above. Any discrepancies were resolved by simultaneous reevaluation of the sections by both observers using a multiheaded microscope.

**Statement of animal care**

All animal experiments have been performed according to the German law for protection of animals and the NIH guidelines for use and care of laboratory animals. All experiments were approved by the Ministerium fuer Landwirtschaft und Naturschutz, Kiel, Germany (V742-72241.121-22 (19-2/05)).

**Statistical evaluation**

Results for disease activity index (DAI) and immunoreactivity score (IRS) were expressed as means ± SEM. Colon length was displayed in Box-Whisker plots indicating median and 5th/95th percentiles. DAI values were analyzed using repeated measures analysis of variance (ANOVA) over all time points. Incidence, grading and growth pattern of adenomas were compared by Fisher’s exact test between both groups, whereas multiplicity of adenomas was illustrated by scatter plot and tested by Mann Whitney test. IRS was analyzed by Kruskal-Wallis test and Dunn’s multiple Comparison test. Comparison of colon lengths between both groups was performed by Mann Whitney test. $P$ values ≤ 0.05 were considered statistically significant and indicated with asterixes (* $P$ ≤ 0.05; ** $P$ ≤ 0.01; *** $P$ ≤ 0.001).

**RESULTS**

**Impact of Taurolidine on disease activity**

As indicated in Figure 1, application of AOM (10 mg/kg body weight) followed by cyclic administration of 0.75% DSS resulted in a tri-phasic colitis with peak DAI values at the end of each DSS treatment interval (i.e. Day 12, 22 and 32, respectively) and recovery during the DSS-free interval [Figure 1]. There were no significant differences in DAI values between the TRD group (TRD 0.2%) and the control group (H$_2$O) during this induction period of 37 days (repeated measures ANOVA over all time points) [Figure 1]. After 100 days, survival was 100% in both groups.

**Macroscopic and microscopic evaluation**

The colon length did not differ significantly between the TRD group (mean 9.1 cm ± 0.2 cm SEM) and the control group (mean 9.1cm ± 0.3cm SEM) (Mann Whitney test). In the control group, 6/9 animals developed adenoma leading
to an incidence of 66.7% compared to 5/9 animals (55.6%) in the TRD group, which was not significantly different (Fisher’s exact test). The multiplicity of adenomas was also not affected by TRD application, since the median number of adenomas per animal between control group (1; range 0-7) and TRD group (2; range 0-4) was not significantly different (Mann Whitney test). In the TRD group as well as in the control group (H₂O), the majority of adenomas was low-grade adenoma (73% vs. 78%) and had a tubular growth pattern (80% vs. 89%) without any significant differences between TRD and control group (Fisher’s exact test). In all animals, inflammatory changes (e.g. mucosal ulceration, crypt loss), aberrant crypt foci (ACF) or adenocarcinoma were absent. Representative histological H and E staining for normal mucosa and tubular adenoma (low grade) of the TRD group and control group (H₂O) are provided in Figure 2.

**Immunoreactivity scores**

After induction of the DSS-AOM model of carcinogenesis (i.e. after 100 days), colonic adenomas as well as normal colonic mucosa were analyzed by immunostaining for different antigens known to be associated with the colitis-dysplasia-carcinoma sequence. Immunoreactivity results were compared between adenoma and normal mucosa as well as between TRD-treated animals (TRD) and the control group (H₂O) [Figure 3].

The expression of the proliferation marker Ki-67 was characterized by a significantly higher expression in adenomatous tissue compared to normal mucosa. Immunoreactivity score (IRS) for Ki-67 in the control group (H₂O) was 2.7 (± 0.2) in adenoma compared to 1.9 (± 0.1) in normal mucosa (P ≤ 0.01). In the TRD group (TRD), Ki-67 score in adenomatous tissue was 2.7 (± 0.1) and in normal mucosa 2.0 (± 0.0) (P ≤ 0.05). There was no significant difference between control group (H₂O) and TRD treatment (TRD) in Ki-67 expression [Figure 3a].

The cell cycle regulator Cyclin-D1 and the transcription factor SOX9 displayed a significantly increased expression in adenomas compared to normal mucosa [Figure 3b, c]. IRS for Cyclin-D1 in adenomas compared to normal mucosa was 3.7 (± 0.1) vs. 1.4 (± 0.2) in the control group (H₂O) compared to 3.7 (± 0.1) vs. 1.9 (± 0.1) in the TRD group (P ≤ 0.001) [Figure 3b]. The overall expression of SOX9 was less pronounced, leading to IRS between 0.7 to 2.0 as indicated in Figure 3c. IRS for SOX9 in adenomas compared to normal mucosa was 1.9 (± 0.1) vs. 0.7 (± 0.1) in the control group (H₂O) (P ≤ 0.01) compared to 2.0 (± 0.1) vs. 0.8 (± 0.1) in the TRD group (P ≤ 0.001) [Figure 3c]. Again, there were no significant differences for both parameters between TRD and control treatment [Figure 3b, c].

E-cadherin showed a heterogeneous expression among both treatment groups [Figure 3d]. Only in the TRD group, IRS for E-cadherin was significantly increased in adenoma (1.2 ± 0.2) compared to normal mucosa (0.3 ± 0.1) (P ≤ 0.001) whereas IRS in the control group (H₂O) did not differ significantly between adenoma (0.8 ± 0.1) and normal mucosa (0.5 ± 0.2) [Figure 3d].

The degree of β-catenin immunoreactivity was highly dependent on its intracellular localization, since β-catenin was only detectable in the cytoplasm [Figure 3e] and in the cell membrane [Figure 3f]. There was no staining of the nucleus. Cytoplasmatic β-catenin was significantly increased in adenomas compared to normal mucosa leading to an IRS of 1.4 (± 0.2) vs. 0.5 (± 0.1) in the control group (H₂O) (P ≤ 0.001) and 1.9 (± 0.1) vs. 0.6 (± 0.1) in the TRD group (P ≤ 0.01) without any significant differences between TRD and control treatment [Figure 3e]. Membranous β-catenin showed a similar expression pattern with elevated IRS in adenomas compared to normal mucosa. However, the differences did not reach statistical significance [Figure 3f].

**Tissue compartment specific results of immunostaining**

Figure 4 displays representative immunostaining of antigens analyzed in this study. Since there were no significant
differences in antigen expression between the TRD group and the control treatment, only photographs of the control group (H₂O) are presented.

In normal mucosa, Ki-67 staining was restricted to the lower part of the crypts, whereas adenomas showed a strong staining of the majority of the cells [Figure 4a, b]. Similarly, Cyclin-D1 and SOX9 immunostaining in normal mucosa was characterized by localized expression in the basal part of the crypts, representing the proliferating compartment [Figure 4c, e]. In contrast, adenomatous tissue displayed a strong immunostaining in the majority of cells [Figure 4d, f]. The cytoplasmatic expression of β-catenin in normal mucosa was limited to scattered superficial cells, whereas
adenoma cells showed a pronounced cytoplasmatic staining [Figure 4g, h].

**DISCUSSION**

In this study, we sought to determine the chemopreventive capacity of oral TRD – a derivate of the aminosulfoacid Taurine – in the DSS-AOM model of carcinogenesis. The rationale for this study was based on two unique characteristics of TRD: its anti-neoplastic and anti-inflammatory properties. TRD has been shown to mediate anti-proliferative and anti-neoplastic effects towards different colon carcinoma cell lines in vitro [19,24,43-47] as well as in vivo in different animal models of colon carcinoma with i.p. [44-46,48-53] or i.v. application [54-58] of TRD. Recently, i.p.-applied TRD has been evaluated in patients undergoing major surgery for CRC. TRD was responsible for reducing cytokines like IL-1β which are regarded as “tumor-stimulating cytokines” – representing a surrogate parameter for metastasis formation. [59] Furthermore, i.v. application of TRD in patients with advanced gastric cancer and glioblastoma showed promising clinical results with almost absent toxicity [27,28] and further oncological trials with i.v. application of TRD are currently being conducted.

The anti-inflammatory action of TRD is caused by its ability to inactivate lipopolysaccharides or exotoxins and to inhibit secretion of different cytokines like TNFα, IL-1 and IL-8, which has been demonstrated in vitro [60,61] and in vivo. [62-64]

This led to a broad spectrum of clinical applications, e.g. prevention and treatment of central venous catheter-related bloodstream infections [16,65] or treatment of peritonitis [66-68].

In a previous study we could recently report that oral application of TRD significantly attenuates chronic DSS colitis in mice leading to significantly reduced disease activity and mortality. [39] Those results were consistent with the finding provided by another group, that i.v. application of TRD reduces disease activity and endotoxemia in the hapten-induced TNBS colitis model in mice. [69]

However, in the DSS-AOM model of carcinogenesis we could not find any chemopreventive effect of TRD. Oral application of 0.2% TRD did not demonstrate any significant chemopreventive effect in terms of incidence, multiplicity, grading or growth pattern of adenoma in the DSS-AOM model of carcinogenesis. Furthermore, there was no difference in the expression of the proliferation marker Ki-67 or the expression of different members of the Wnt signaling pathway (Cyclin-D1, β-catenin, E-cadherin or SOX9) between TRD-treated animals and the control group. Considering the strong clinical amelioration we encountered previously with oral application of TRD 0.2% in the chronic DSS colitis model, [39] one reason for the missing chemopreventive effect could be the oral application of TRD, that might not be sufficient to exert anti-proliferative activity. Another reason could be that TRD interferes with inflammatory cytokines that are sometimes active in malignant tumors. In contrast to such tumors as well as in contrast to severe colitis, after 100 days, here, the disease activity as well as aberrant crypt foci were absent. We regard the chemopreventive effect of TRD as less relevant as it could be expected from previous studies. [39]

Besides studying the chemopreventive effect of TRD in the DSS-AOM model of carcinogenesis, we tried to gain further insight into the experimental model itself. [132] Hence, we analyzed by immunohistochemistry some components of the Wnt signal transduction pathway, which has been identified as one of the key pathways in the initiation and development of CRC. [70,71] as well as in the DSS-AOM model of carcinogenesis. [72-75] β-catenin – a crucial downstream effector of the Wnt signaling pathway – showed a significantly higher
cytoplasmatic expression in adenomas compared to normal mucosa whereas the membranous expression was almost unaffected by the application of DSS-AOM. This observation is supported by other authors, who similarly encountered a cytosolic accumulation of β-catenin in adenomas as well as in adenocarcinomas using the same experimental model. In human colon cancer, the cytosolic accumulation of β-catenin is either caused by mutational inactivation of the APC tumor suppressor gene or by mutation of β-catenin itself leading to nuclear translocation and binding to TCF4 (T-cell Factor 4). The β-catenin/TCF4 complex activates transcription of several oncogenic genes. We analyzed two important target genes of the β-catenin/TCF4 complex that have not been described so far in the DSS-AOM model: Cyclin-D1 and SOX9. Cyclin-D1 represents another effector of Wnt signaling and important target gene of β-catenin in the intestine. SOX9 is a member of the SOX (SRY box = sex determining region Y box) gene superfamilies of transcription factors and is characterized by a highly conserved high-mobility group (HMG) DNA-binding domain. SOX9 has been shown to be involved in the development and differentiation of many cell types and tissues, e.g. chondrocytes, pancreatic tissue, prostate, testis as well as melanocytes. Furthermore, SOX9 is crucial for the development of intestinal epithelium since inactivation of SOX9 results in severe defects in differentiation of Paneth and Goblet cells. There is growing evidence that SOX9 also plays an important role in different malignancies e.g. prostate, brain or breast cancer. Recently, it has been shown that SOX9 is highly overexpressed in CRC and overexpression is significantly associated with a lower five-year survival. So far SOX9 expression has neither been reported in human UC-associated CRC or animal models like the DSS-AOM model of carcinogenesis. In our study, adenomas were characterized by significantly higher SOX9 expression compared to normal mucosa suggesting an important role in this paradigm of UC-associated CRC.

CONCLUSIONS

The anti-inflammatory and anti-neoplastic substance Taurolidine did not show any chemopreventive capacity in the DSS-AOM model of carcinogenesis. There was no difference in adenoma formation and biology between TRD-treated animals and untreated animals. However, the expression of SOX9 and Cyclin-D1 – two key players of the Wnt signaling pathway – has been described for the first time in this model for UC-associated CRC.

REFERENCES

1. Lakatos PL, Lakatos L. Risk for colorectal cancer in ulcerative colitis: Changes, causes and management strategies. World J Gastroenterol 2008;14:3937-47.
2. Munkholm P. Review article: The incidence and prevalence of colorectal cancer in inflammatory bowel disease. Aliment Pharmacol Ther 2003;18:1-5.
3. Zisman TL, Rubin DT. Colorectal cancer and dysplasia in inflammatory bowel disease. World J Gastroenterol 2008;14:2462-9.
4. Rutter MD, Saunders BP, Wilkinson KH, Rumbles S, Schofield G, Kamm MA, et al. Thirty-year analysis of a colonoscopic surveillance program for neoplasia in ulcerative colitis. Gastroenterology 2006;130:1030-8.
5. Eaden JA, Abrams KR, Mayberry JF. The risk of colorectal cancer in ulcerative colitis: A meta-analysis. Gut 2001:48:526-35.
6. Levine JS, Burakoff RJ. Chemoprophylaxis of colorectal cancer in inflammatory bowel disease: Current concepts. Inflamm Bowel Dis 2007;13:1293-8.
7. Das D, Arber N, Jankowski JA. Chemoprevention of colorectal cancer. Digestion 2007;76:51-67.
8. Tung BY, Emond MJ, Haggitt RC, Bronner MP, Kimmey MB, Kowdley KV, et al. Ursodiol use is associated with lower prevalence of colonic neoplasia in patients with ulcerative colitis and primary sclerosing cholangitis. Ann Intern Med 2001;134:89-95.
9. Pardi DS, Loftus EV Jr, Kremers WK, Keach J, Lindor KD. Ursodeoxycholic acid as a chemopreventive agent in patients with ulcerative colitis and primary sclerosing cholangitis. Gastroenterology 2003;124:889-93.
10. Velayos FS, Terdiman JP, Walsh JM. Effect of 5-aminosalicylate use on colorectal cancer and dysplasia risk: A systematic review and meta-analysis of observational studies. Am J Gastroenterol 2005;100:1345-53.
11. Andrews JM, Travis SP, Gibson PR, Gasche C. Systematic review: Does concurrent therapy with 5-ASA and immunomodulators in inflammatory bowel disease improve outcomes? Aliment Pharmacol Ther 2009;29:459-69.
12. Bernstein CN, Blanchard JF, Mete C, Yogendran M. Does the use of 5-aminosalicylates in inflammatory bowel disease prevent the development of colorectal cancer? Am J Gastroenterol 2003;98:2784-8.
13. Bernstein CN, Eaden J, Steinhart AH, Munkholm P, Gordon PH. Cancer prevention in inflammatory bowel disease and the chemoprophylactic potential of 5-aminosalicylic acid. Inflamm Bowel Dis 2002;8:356-61.
14. Allison M. Dialysis catheter-related bacteremia: Treatment and prophylaxis. Am J Kidney Dis 2004;44:779-91.
15. Reith HB. [Therapy of peritonitis today: Surgical management and adjuvant therapy strategies]. Langenbecks Arch Chir 1997;382:514-7.
16. Koldenhoff M, Zakrzewski JL. Taurolidine is effective in the treatment of central venous catheter-related bloodstream infections in cancer patients. Int J Antimicrob Agents 2004;24:491-5.
17. Calabresi P, Goulette FA, Darnowski JW. Taurolidine: Cytotoxic and mechanistic evaluation of a novel antineoplastic agent. Cancer Res 2001;61:6816-21.
18. Jacobi CA, Menenakos C, Braumann C, Tauroidine: A new drug with anti-tumor and anti-angiogenic effects. Anticancer Drugs 2005;16:917-21.
19. Chromik AM, Daigelger A, Hilgert C, Bulut D, Geisler A, Liu V, et al. Synergistic effects in apoptosis induced by taurolidine and TRAIL in HCT-116 colon carcinoma cells. J Invest Surg 2007;20:339-48.
20. Daigelger A, Chromik AM, Geisler A, Bulut D, Hilgert C, Krieg A, et al. Synergistic apoptotic effects of taurolidine and TRAIL on squamous carcinoma cells of the esophagus. Int J Oncol 2008;32:1205-20.
21. Daigeler A, Brenzel C, Bulut D, Gessler A, Hilgert C, Lohhardt M, et al. TRAIL and Taurolidine induce apoptosis and decrease proliferation in human fibrosarcoma. J Exp Clin Cancer Res 2008;27:82.

22. Han Z, Ribbia I, Pantazis PW, Yche J, Darnowski J, Calabrese P. The antibacterial drug taurolidine induces apoptosis by a mitochondrial cytochrome c-dependent mechanism. Anticancer Res 2002;22:1959-64.

23. Darnowski JW, Goulette FA, Cozens LP, Chatterjee D, Calabrese P. Mechanistic and antineoplastic evaluation of taurolidine in the DU145 model of human prostate cancer. Cancer Chemother Pharmacol 2004;54:249-38.

24. Braumann C, Henke W, Jacobi CA, Dubiel WT. The tumor-suppressive reagent taurolidine is an inhibitor of protein biosynthesis. Int J Cancer 2004;i:1225-30.

25. Rodak R, Kubota H, Ishihara H, Eguster HP, Konu D, Mohler H, et al. Induction of reactive oxygen intermediates-dependent programmed cell death in human malignant ex vivo glioma cells and inhibition of the vascular endothelial growth factor production by taurolidine. J Neurosurg. 2005;102:1055-68.

26. Stendel R, Biever HR, Dekany GM, Kubota H, Munz C, Wang S, et al. The antibacterial substance taurolidine exhibits anti-neoplastic action based on a mixed type of programmed cell death. Autophagy 2009;i:194-210.

27. Braumann C, Winkler G, Rogalla P, Menenakos C, Jacobi CA. Prevention of disease progression in a patient with a gastric cancer-recurrence: Outcome after intraperitoneal treatment with the novel antineoplastic agent taurolidine: Report of a case. World J Surg Oncol 2006;4:34.

28. Stendel R, Picht T, Schlilling A, Heidenreich J, Loddkenkemper C, Janisch W, et al. Treatment of glialbloma with intravenous taurolidine: First clinical experience. Anticancer Res 2004;i:1143-7.

29. Clapper ML, Cooper HS, Chang WC. Dextran sulfate sodium-induced colitis-associated neoplasia: A promising model for the development of chemopreventive interventions. Acta Pharmcolog Sin 2007;28:1450-9.

30. Boivin GP, Washington K, Yang K, Ward JM, Pretlow TP, Russell R, et al. Susceptibility to azoxymethane and dextran sodium sulfate. Cancer Sci 2004;95:721-7.

31. Tanaka T, Kohno H, Suzuki R, Shim H, Shah RS, Ibrahim SA, Sedergran DJ. Treatment of peritoneal tumor cell growth and implantation in a colon cancer rat model. Eur Surg Res 2007;39:129-35.

32. Tanaka T. Colorectal carcinogenesis: Review of human and experimental animal studies. J Carcinog 2009;i:8.

33. Yasui Y, Tanaka T. Protein expression analysis of inflammation-related colon cancerogenesis. J Carcinog 2009;i:10.

34. Cooper HS, Murthy SN, Shah RS, Sedergran DJ. Clinopathologic study of dextran sulfate sodium experimental murine colitis. Lab Invest 1993;i:69:238-49.

35. Murthy SN, Cooper HS, Shin H, Shah RS, Ibrahim SA, Sedergran DJ. Treatment of dextran sulfate sodium-induced murine colitis by intracolonic cyclosporin. Dig Dis Sci 1993;i:38:172-34.

36. Suzuki R, Kohno H, Sugie S, Tanaka T. Sequential observations on the occurrence of preneoplastic and neoplastic lesions in mouse colon treated with azoxymethane and dextran sodium sulfate. Cancer Sci 2004;i:95:721-7.

37. Suzuki R, Kohno H, Sugie S, Tanaka T. Dose-dependent promoting effect of dextran sulfate sodium on mouse colon carcinogenesis initiated with azoxymethane. Histol Histopathol 2005;20:483-92.

38. Suzuki R, Kohno H, Sugie S, Nakagama H, Tanaka T. Strain differences in the susceptibility to azoxymethane and dextran sodium sulfate-induced colon cancerogenesis in mice. Carcinogenesis 2006;i:27:162-9.

39. Chromik AM, Muller AM, Albrecht M, Rottmann S, Otto JM, Herdegen T, et al. Oral administration of taurolidine ameliorates chronic DSS colitis in mice. J Invest Surg 2007;i:20:273-82.

40. Chromik AM, Muller AM, Korner J, Belyaev Q, Holland-Letz T, Schmitz F, et al. Genetic deletion of JNK1 and JNK2 aggravates the DSS-induced colitis in mice. J Invest Surg 2007;i:20:23-33.

41. Remmele W, Stegner HE. [Recommendation for uniform definition of an immuno-reactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue]. Pathologe 1987;i:18:138-40.

42. Lyall MS, Dunsard SR, Curran S, Murray GI. Profiling markers of prognosis in colorectal cancer. Clin Cancer Res 2006;i:12:1184-91.

43. Jacobi CA, Ordemann J, Bohm B, Zieren HU, Sabat R, Muller JM. Inhibition of peritoneal tumor cell growth and implantation in laparoscopic surgery in a rat model. Am J Surg 1997;i:174:359-63.

44. McCourt M, Wang JH, Sookhai S, Redmond HP. Taurolidine inhibits tumor cell growth in vitro and in vivo. Ann Surg Oncol 2000;i:7:685-91.

45. Nestler G, Schulz HJ, Schubert D, Kruger S, Lippert H, Pross M. Impact of taurolidine on the growth of C33A colon carcinoma cells in vitro and in a laparoscopic animal model in rats. Surg Endosc 2005;i:19:2280-4.

46. Jacobi CA, Peter FJ, Wenger FA, Ordemann J, Muller JM. New therapeutic strategies to avoid intra- and extraperitoneal metastases during laparoscopy: Results of a tumor model in the rat. Dig Surg 1999;i:16:393-9.

47. Chromik AM, Daigeler A, Bulut D, Flier A, May C, Harati K, et al. Comparative analysis of cell death induction by Taurolidine in different malignant human cancer cell lines. J Exp Clin Cancer Res 2010;i:29:21.

48. Opitz I, van der Veen H, Witsie P, Braumann C, Muller JM. Influence of different gases and intraperitoneal instillation of an antineoplastic agent on peritoneal tumor cell growth and implantation with laparoscopic surgery in a rat model. Surg Endosc 1999;i:13:1021-5.

49. Opitz I, van der Veen H, Braumann C, Muller JM. Inhibition of peritoneal tumor cell growth and implantation in laparoscopic surgery in a rat model. Am J Surg 1997;i:174:359-63.

50. Bobrich E, Braumann C, Opitz I, Menenakos C, Kristiansen G, Jacobi CA. Influence of intraperitoneal application of taurolidine/heparin on expression of adhesion molecules and colon cancer in rats undergoing laparoscopy. J Surg Res 2007;i:137:75-82.

51. Wittich P, Meieradji A, Marquet RL, Bonjer HJ. Irrigation of port sites: Prevention of port site metastases? J Laparoendosc Adv Surg Tech A 2004;i:14:125-9.

52. Hokscha B, Ruler B, Gazdhar A, Bilić M, Beshay M, Gugger M, et al. Taurolidine in the prevention and therapy of lung metastases. Eur J Cardiothorac Surg 2009;i:36:058-63.

53. Braumann C, Schoenbeck M, Menenakos C, Kilian M, Jacobi CA. Effects of increasing doses of a bolus injection and an intravenous long-term therapy of taurolidine on subcutaneous (metastatic) tumor growth in rats. Clin Exp Metastasis 2005;i:22:77-83.

54. Braumann C, Stuhldreier B, Bobrich E, Menenakos C, Rogalla S, Jacobi CA. High doses of taurolidine inhibit advanced intraperitoneal tumor growth in rats. J Surg Res 2005;i:129:129-35.

55. Braumann C, Ordemann J, Kilian M, Wenger FA, Jacobi CA. Local and systemic chemotherapy with taurolidine and taurolidine/heparin in colon cancer-bearing rats undergoing laparotomy. Clin Exp Metastasis 2003;i:20:387-94.

56. Braumann C, Ordemann J, Wildbrett PJ, Jacobi CA. Influence of intraperitoneal and systemic application of taurolidine and taurolidine/heparin during laparoscopy on intraperitoneal and subcutaneous tumour growth in rats. Clin Exp Metastasis 2000:i:18:547-52.

57. Braumann C, Gutt CN, Scheele J, Menenakos C, Willems W, Muller JM, et al. Taurolidine reduces the tumor stimulating cytokine interleukin-1-beta in patients with resectable gastrointestinal cancer: A multicentre prospective randomized trial. World J Surg Oncol 2009;i:7:32.

58. Dofferhoff AS, Esselink MT, de Vries-Hospers HG, van Zanten A, Bom VJ, Weits J, et al. The release of endotoxin from antibiotic-treated Escherichia coli and the production of tumour necrosis factor by human monocytes. J Antimicrob Chemother 1993;i:31:373-84.

59. Bedrosian I, Sofia RD, Wolff SM, Dinarello CA. Taurolidine, an analogue of the amino acid taurine, suppresses interleukin I and tumor necrosis factor synthesis in human peripheral blood mononuclear cells. Cytokine 1991;i:3:668-75.

60. Rosman C, Westerveld GJ, Kooi K, Bleichrodt RP. Local treatment of generalised peritonitis in rats: effects on bacteria, endotoxin and mortality. Eur J Surg 1999;i:165:1072-9.

61. Rosman C, Westerveld GJ, van Oeveren W, Kooi K, Bleichrodt RP. Effect of intraperitoneal antimicrobials on the concentration of bacteria, endotoxin, and tumor necrosis factor in abdominal fluid and plasma in rats. Eur Surg Res 1996;i:28:351-60.

62. Watson RW, Redmond HP, Mc Carthy J, Bouchier-Hayes D. Taurolidine, an
antilipopolysaccharide agent, has immunoregulatory properties that are mediated by the amino acid tyrosine. J Leukoc Biol 1995;58:299-306.

65. Simon A, Ammann RA, Witzenshreyder G, Bode U, Fleischhacker G, Besuden MM. Taurodilin-citrate lock solution (TauroLock) significantly reduces CVAD-associated graftversus-host disease in pediatric cancer patients. BMC Infect Dis 2008;8:102.

66. Linder MM, Ott W, Wesch G, Wicki O, Marti MC, Moser G. [Therapy of purulent peritonitis: Documentation of 78 cases and experience with taurolin (author’s transl)]. Langenbecks Arch Chir 1981;353:241-50.

67. Browne MK. The treatment of peritonitis by an antiseptic - taurolin. Pharmacotherapeutics 1981;2:517-22.

68. Wesch G, Petermann C, Linder MM. [Drug therapy of peritonitis: 6-year experience with the chemotherapeutic agent and anti-endotoxin taurolin]. Fortschr Med 1983;101:545-50.

69. Gardiner KR, Anderson NH, McCaigue MD, Erwin PJ, Halliday MI, Rowlands BJ. Enteral and parenteral anti-endotoxin treatment in experimental colitis. Hepatogastroenterology 1994;41:554-8.

70. Kolls JG, Bommier G, Goke B. Wnt/beta-catenin/tcf signaling: A critical pathway in gastrointestinal tumorigenesis. Digestion 2002;66:131-44.

71. Oving IM, Clevers HC. Molecular causes of colon cancer. Eur J Clin Invest 2002;32:448-57.

72. Cooper HS, Murthy S, Kido K, Yoshitake H, Flanigan A. Dysplasia and cancer-associated neoplasia in the human: A study of histopathology. B-catenin and p53 expression and the role of inflammation. Cancer Research 2000;61:757-68.

73. Tanaka T, Kohno H, Suzuki R, Sugie S, Mori H. A novel inflammation-related mouse colon cancerogenesis model induced by azoxymethane and dextran sulfate sodium. Cancer Sci 2003;94:965-73.

74. Fujii S, Fujimori T, Kawamata H, Takeda J, Kitajima K, Momotaehara F, et al. Surgical oncology: Inflammatory bowel disease, sepsis, clinical ethics consultation. Cancer Sci 2003;94:56-63.

75. Kohno H, Suzuki R, Sugie S, Tanaka T, Suppression of colitis-associated neoplasia by a COX-2 inhibitor and PPAR ligands. BMC Cancer 2005;5:46.

76. Behrens J. Control of beta-catenin signaling in tumor development. Ann N Y Acad Sci 2000;910:21-33; discussion 33-5.

77. Kim JK, Diehl JA. Nuclear cyclin D1: An oncogenic driver in human cancer. J Cell Physiol 2009;220:292-6.

78. Behrens J, Diehl JA. Nuclear cyclin D1: An oncogenic driver in human cancer. J Cell Physiol 2009;220:292-6.

79. Assi K, Mills J, Owen D, Org C, St Arnau D, Reddah S, et al. Integrin-linked kinase regulates cell proliferation and tumour growth in murine colitis-associated carcinogenesis. Gut 2008;57:931-40.

80. Blache P, van de Wetering M, Duluc D, Domen G, Berta P, Freudent JN, et al. SOX9 is an intestine crypt transcription factor, is regulated by the Wnt pathway, and represses the CDX2 and MUC2 genes. J Cell Biol 2004;166:37-47.

81. Yano F, Kugimiyah F, Ohba S, Ikeda T, Chikuda H, Ogasaewara T, et al. The canonical Wnt signaling pathway promotes chondrocyte differentiation in a Sox9-dependent manner. Biochem Biophys Res Commun 2005;333:1300-8.

82. Akiyama H, Lyons JP, Mori-Akiyama Y, Yang X, Zhang R, Zhang Z, et al. Interactions between Sox9 and beta-catenin control chondrocyte differentiation. Genes Dev 2004;18:1072-87.

83. de Crombrugghe B, Lefebvre P, Brehirer RR, Brehirer RR, Brehirer RR, Brehirer RR. FOXP4 is a key player in ultraviolet B-induced melanocyte differentiation and pigmentation. Proc Natl Acad Sci U S A 2007;104:13984-9.

84. Lynn FC, Smith SB, Wilson ME, Yang KY, Nekreps N, German MS. Sox9 coordinates a transcriptional network in pancreatic progenitor cells. Proc Natl Acad Sci U S A 2007;104:10500-5.

85. Thomsen MK, Francis JC, Swain A. The role of Sox9 in prostate development. Differentiation 2008;76:728-735.

86. Kobayashi A, Chang H, Chaboisier MC, Schied A, Beheringer RR, Sox9 in testis determination. Ann N Y Acad Sci 2005;1061:19-7.

87. Passerone T, Valencia JC, Bortolotto C, Hoashi T, Le Pape E, Takahashi K, et al. SOX9 is a key player in ultraviolet B-induced melanocyte differentiation and pigmentation. Proc Natl Acad Sci U S A 2007;104:13984-9.

88. Bastide P, Danaro C, Pannequin J, Kist R, Robine S, Marty-Double C, et al. Sox9 regulates cell proliferation and is required for Paneth cell differentiation in the intestinal epithelium. J Cell Biol 2007;178:635-48.

89. de Bont JM, Kros JM, Passier MM, Reddingius RE, Sillesv Smitt PA, Luder TM, et al. Differential expression and prognostic significance of SOX genes in pediatric medulloblastoma and ependymoma identified by microarray analysis. Neuro Oncol 2008;10:648-60.

90. Endo Y, Deonauth K, Prahalat F, Hojter B, Zhu Y, Byers SW. Role of Sox-9, ErbB1 and VEGF-activated extracellular matrix signaling in breast cancer cell. PLoS One 2008;3:e2714.

91. Wang H, Leav I, Ibaragi S, Wegner M, Hu GF, Lu ML, et al SOX9 is expressed in human fetal prostate epithelium and enhances prostate cancer invasion. Cancer Res 2008;68:1625-30.

92. Liu B, Xu J, Lai M, Zhang H, Chen J. A transcriptome analysis of human colorectal cancers. BMC Cancer 2006;6:40.

93. Liu B, Fang Y, Xu J, Wang L, Xu F, Xu E, et al. Analysis of SOX9 expression in colorectal cancer. Am J Clin Pathol 2008;130:897-904.

**AUTHOR’S PROFILE**

Dr. Ansgar Michael Chromik PERSONAL DETAILS

Name Ansgar Michael Chromik, MD Date of birth September 5th, 1974 Place of birth Kiel, Germany FIELDS OF INTEREST Research: Apoptosis, surgical oncology, Inflammatory bowel disease, sepsis, clinical ethics consultation CURRENT POSITIONS Since 2007 Attending Surgeon in the Dept. of General and Visceral Surgery, St. Josef-Hospital, Ruhr University of Bochum Germany PREVIOUS POSITIONS 2004-2007 Residency in General Surgery at the Surgical Department, St. Josef-Hospital, Ruhr University of Bochum Germany 2002-2004 Residency in General Surgery at the Surgical Department, University Hospital of Münster, Germany EDUCATION 2007 Board Certification in “General Surgery” 2003 Doctoral degree “magna cum laude” in medicine, University of Kiel, Germany 2002 Final examination in medicine at the Humboldt University of Berlin, Germany 2002 USMLE, Step II.

Journal of Carcinogenesis is published by Carcinogenesis Press by Medknow Publications and Media Pvt. Ltd.

Manuscripts submitted to the journal are peer reviewed and published immediately upon acceptance, cited in PubMed and archived on PubMed Central. Your research papers will be available free of charge to the entire biomedical community. Submit your next manuscript to Journal of Carcinogenesis.

www.journalonweb.com/jcar