Promising cytotoxic activity profile of fermented wheat germ extract (Avemar®) in human cancer cell lines

W. Voigt, T. Mueller, K. Jordan, F. Reipsch, K. Nerger and H.-J. Schmoll
Department of Hematology/Oncology, Halle/Saale, Germany

Martin-Luther-Universität Halle-Wittenberg

Abstract

Avemar® is a fermented wheat germ extract (FWGE) with potent antimetastatic, antiproliferative and immunomodulatory activities. Chemically, it is a complex mixture of biologically active molecules including 2-methoxy-p-benzoquinone and 2,6-dimethoxy-p-benzoquinone which were supposed to be responsible for the main biological properties of Avemar. Despite its ubiquitous use as nutrition supplement for cancer patients in some countries only limited data are available on its activity in human cancer or in combination with chemotherapy. Aim of this study was to investigate the potential activity of Avemar in a panel of human cancer cell lines including colon, testis, thyroid, ovary, NSCLC, breast, gastric, Head and Neck, hepatoma, glioblastoma, melanoma, cervix and neuroblastoma and to rule out antagonism with conventional chemotherapy. To asses the cytotoxic activity of a 96 h continuous drug exposure of Avemar alone or in combination with 5-FU, Oxaliplatin or CPT-11 the sulforhodamine B assay was used and drug interaction between Avemar and cytostatic drugs was analyzed by the method of Drewinko.

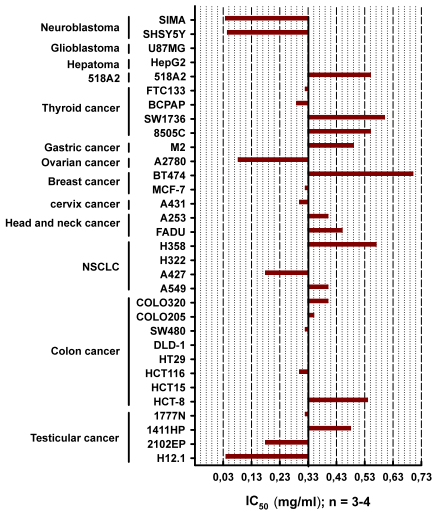
IC50 of Avemar ranged from 0.038 mg/ml to 0.7 mg/ml with a median IC50 of 0.33 mg/ml. The highest activity was found in neuroblastoma cell lines with an average IC50 of 0.042 mg/ml. Of note, the 8 colon cancer cell lines included in this screen had a very narrow IC50 range ranging from 0.3 mg/ml to 0.54 mg/ml.

Parallel drug treatment with Avemar and either 5-FU, Oxaliplatin or CPT-11 in colon cancer cell lines exerted additive to synergistic effects for all drugs with the highest degree of synergy found for combinations of Avemar with 5-FU. No antagonistic drug interaction was observed for parallel drug exposure. Currently, the relevance of sequential treatment for drug combinations with Avemar is analyzed in colon cancer cell lines and the potential differentiating property of Avemar is investigated in testicular cancer cell lines using cellular morphology and Oct-4 protein expression as marker for differentiation.

In conclusion, Avemar posses broad spectrum preclinical antineoplastic activity and additive to synergistic drug interactions were observed for combinations with CPT-11, Oxaliplatin and 5-FU in colon cancer cell lines.

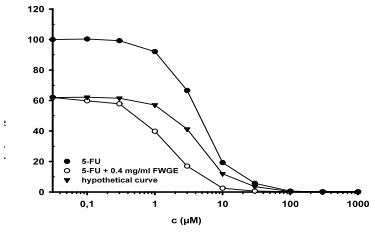
Further evaluation of Avemar as potential anticancer agent seems warranted. Combined treatment of colorectal cancer patients with CPT-11 or Oxaliplatin containing regimens and Avemar seems feasible with respect to drug interaction on the cellular level.

Fig. 1 In vitro cytotoxic activity of fermented wheat germ extract



Legend: IC50 of at least 3 independent experiments per cell line were averaged and summarized as a mean graph for better comparison. The median IC50 is 0.33 mg/ml. The highest activity of fermented wheat germ extract was found on neuroblastoma and ovarian cancer cell lines. It's interesting to note that the IC50-values of the majority of CRC cell lines included in this screen range close to the median IC50.

Fig. 2 Synergy between fermented wheat germ extract and 5-FU in human colon cancer cell line HCT15



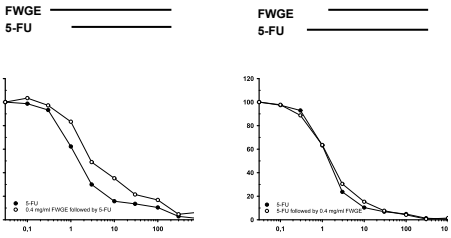
Legend: HCT15 cells were exposed to 5-FU and Avemar in parallel continuously for 96 h. Plots represent the average of 3 independent experiments. Synergy is indicated by the hypothetical curve which runs above the combination curve.

Tab. 1 Drug interaction between fermented wheat germ extract and either 5-FU, Oxaliplatin or CPT-11 (parallel exposure)

	IC50 (µM)								
Cell line	Oxaliplatin ± Avemar		p-value	5-FU ± Avemar		p-value	CPT-11 ± Avemar		p-value
	-	+		-	+		-	+	
HCT-8	0,43±0,03	0,45±0,03	0,52	2,65±0,35	1,2±0,6	0,023*	2±0,46	1,8±0,32	0,63
HCT-15	0,95±0,19	0,57±0,25	0,05	4,45±0,72	1,45±0,61	0,0001*	4,5±0,3	3,4±0,31	0,001*
HCT116	0,39±0,06	0,19± 0,09	0,01*	4,6±0,38	2,9±0,9	0,01*	1,2±0,1	0,96±0,11	0,01*
HT29	0,32±0,09	0,35±0,05	0,53	0,99±0,31	1,3±0,6	0,39	3,5±0,3	4,1±0,23	0,05
DLD-1	2,47±0,17	2,2±0,8	0,61	3,2±0,21	1,6±0,7	0,02*	6,6±0,6	6,1±0,85	0,43
Colo205	0,45±0,05	0,24±0,05	0,001*	0,54±0,12	0,44±0,1	0,26	1,2±0,19	1,1±0,19	0,24
Colo320	1,1±0,34	0,84±0,13	0,33	1,35±0,133	0,57±0,03	0,001*	8,5±3,4	8,7±3,1	0,92
SW48	0,13±0,02	0,1±0,02	0,09	0,35±0,02	0,22±0,02	0,0003*	2,4±0,35	2,1±0,29	0,18
SW480	0,57±0,11	0,37±0,12	0,06	0,27±0,09	0,26±0,13	0,83	6,4±1,2	6,9±2,3	0,72

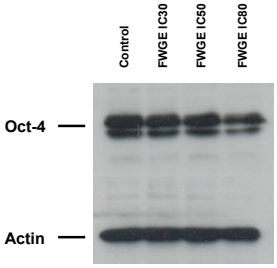
n ≥ 3, asterisk indicates significant synergistic drug interaction

Fig. 3 Sequential application of fermented wheat germ extract and 5-FU in colon cancer cell line HCT-8



Legend: IC30 of fermented wheat germ extract (FWGE) was combined with 5-FU. FWGE was either added 24 h before start of 5-FU drug exposure or 24 h after start of 5-FU drug exposure. Plots represent the average of 2 independent experiments. For better illustration of drug interaction, dose response to 5-FU of the combination curve was normalized to the FWGE treated control. Note that drug sequence influences the way of drug interaction. If FWGE precedes 5-FU for 24 h, drug interaction was antagonistic. If 5-FU precedes FWGE drug was found additive.

Fig. 4 Expression of Oct-4 protein as a marker of cellular differentiation in testicular cancer cell line H12.1



Legend: To assess the differentiating properties of fermented wheat germ extract (FWGE) H12.1 cells were seeded at low density in RPMI 1640 supplemented with 2 % FBS. After 24 h, FWGE was added at the indicated concentrations and cells were exposed for 120 h. Adherent cells were harvested and protein was extracted with RIPA-buffer. Western blot for Oct-4 was performed as recently published (Mueller et al., Tumor Biology, 2006). Higher drug concentrations of FWGE reduced the protein expression of Oct-4 which suggest the initiation of cellular differentiation by FWGE.

Conclusions:

- **FWGE exerted broad spectrum preclinical antineoplastic activity. The highest activity was observed in neuroblastoma, testicular cancer and ovarian cancer cell lines.**
- **Parallel drug exposure of FWGE and 5-FU yielded mainly synergistic effects in colon cancer cell lines (6/8). Mainly additive drug interaction was found for the continuous exposure of FWGE and Oxaliplatin or CPT-11 in colon cancer cell lines.**
- **Scheduling data of 5-FU and FWGE suggest an influence of drug sequence on drug interaction ranging from synergy to antagonism. Based on the data available so far, the most promising schedule for the combination of 5-FU and FWGE is parallel drug exposure which yielded mainly synergistic drug interaction.**
- **At higher drug concentrations (IC80) FWGE appeared to initiate cellular differentiation which is indicated by the loss of Oct-4 expression in H12.1 cell line.**
- **Further research is warranted to clarify the potential role of FWGE as an anticancer drug.**