The steroid sulfatase inhibitor BN83495 inhibits E1S-stimulated growth of DMBA-induced mammary tumours in rat

#163

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ABSTRACT

Steroid sulfatase (STS) is a new target for the treatment of steroid hormone-dependent diseases such as breast, prostate or endometrial cancer. In breast cancer, estrogens play a major role in the establishment of the disease and between one to two-thirds of tumours are estrogen receptor (ER) positive. Despite current hormonal treatments, improvement is still required to achieve better disease control and improve disease outcome. BN83495 is a non-steroidal, non-estrogenic, potent, irreversible STS inhibitor that blocks both the formation of E1 from estrone sulphate and androstenediol from DHEAS. The ability of BN83495 to inhibit E1S-stimulated tumour growth in the rat was examined in a DMBA-induced mammary tumour model. Based on median tumour volume and the interquartile range at the end of the treatment period, BN83495 displayed the greatest antitumour activity compared to TAMOXIFEN or FULVESTRANT. Addition of FULVESTRANT or TAMOXIFEN to BN83495 did not improve the potent antitumour activity observed with BN83495 alone. Pharmacokinetic data of BN83945 and effects on estradiol levels are discussed. Altogether, these preclinical results have supported the entry of BN83945 into further clinical trials for estrogen receptor-positive breast cancer patients.

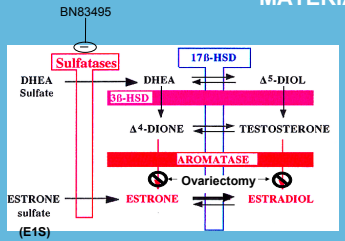
INTRODUCTION

• Estradiol (E2) is considered to be the main growth factor for estradiol receptor positive breast cancer. Several drugs developed to block the activity of estradiol are approved for clinical use. The two main strategies to design these drugs are: 1) reducing the biosynthesis of estradiol to decrease its concentration in the tumour and in the plasma (aromatase inhibitors, LHRH analogs) and 2) inhibiting the direct binding to the estradiol receptor (partial agonist, pure antagonist). However, despite advances in early detection and the understanding of the molecular basis of breast cancer biology, after a variable period of time, recurrent disease is observed. At that point of resistance to therapy, novel hormonal strategies are required.

• Steroid sulfatase (STS) is an enzyme widely distributed and responsible for the hydrolysis of aryl and alkyl steroid sulphates. The development of new steroid sulfatase inhibitors by inhibiting the production of estradiol and androstenediol derived from sulphated precursors (Estrone sulphate, DHEA sulphate) may represent a new important therapeutic strategy for the treatment of postmenopausal women with breast cancer .

• In this poster, using the DMBA-induced mammary tumour model, we found that BN83495 caused regression of estrone sulphate-stimulated tumour growth and showed that its efficacy compared well to both inhibitors of estradiol receptors TAMOXIFEN and FULVESTRANT.

MATERIALS AND METHODS



- One hundred and thirty five (135) Sprague-Dawley rats received a single *PO* administration of DMBA at 20 mg DMBA/rat (1).
- The rats were randomized at D103 when 50% of rats bore at least one tumour with a minimum volume of 500 mm^3 in 8 groups of 9 rats.
- One day after the randomization (D104), 63 rats from groups 2 to 8 were ovariectomised.
- The E1S treatment started 24 hours after ovariectomy (D105). Fifty four (54) rats from groups 3 to 8 received one daily SC injection of E1S from D105 to D162.
- Blood Samples were collected at D118 for estradiol level measurement and at D132 to determine the pharmacokinetic profile of BN83495 and IDP17619 (BN83495 metabolite).
- Rat's viability and behaviour were recorded every day. The body weight of rats and tumour growth was recorded once a week. Isofluran forene (Minerve, Bondoufle, France) was used to anaesthetize the animals before SC treatment, ovariectomy, palpation (and tumour measurements) and termination. Representative curves: cumulative tumour volume (number of tumour *per* rat between 1 and 13). Animal experiments were performed according to ethical guidelines of animal experimentation (2) and the English guidelines for welfare of animals in experimental neoplasia (3). All procedures with animals were submitted to the Animal Care and Use Committee of Pharmacy and Medicine University (Dijon).

1. ZACCHEO T. *et al*., Eur. J. Cancer, 27: 1145-1150, 1991.
2. Principe d'éthique de l'expérimentation animale. Directive n°86/609 CEE du 24 Nov. 1986, Décrêt n°87/848 du 19 Oct. 1987, Arrêté d'Application du 19 Avril 1988,
3. WORKMAN P. *et al., UKCCCR guideline*, Br. J. Cancer, 77: 1-10, 1998.

Treatment schedule

Groups	No animals	Ovariectomy	E1S	Treatment	Dose (mg/kg/adm)	Route	Treat. schedule	Combined Treatment	Dose (mg/kg/adm)	Route	Treat. schedule
1	9	No	No	-	-	-	-	-	-	-	
2	9	Yes	No	-	-	-	-	-	-	-	-
3	9	Yes	Yes	Vehicle	-	*PO*	Q1Dx31	-	-	-	-
4	9	Yes	Yes	-	-	-	-	TAMOXIFEN	10	*PO*	Q1Dx31
5	9	Yes	Yes					FULVESTRANT	50	IM	TWx9
6	9	Yes	Yes	BN83495	100	*PO*	Q1Dx31	-	-	-	-
7	9	Yes	Yes	BN83495	100	*PO*	Q1Dx31	TAMOXIFEN	10	*PO*	Q1Dx31
8	9	Yes	Yes	BN83495	100	*PO*	Q1Dx31	FULVESTRANT	50	IM	TWx9

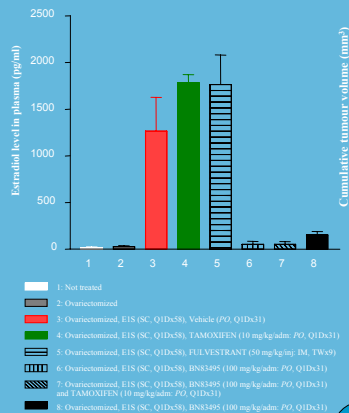
PO: Per Os
Q1Dx31: One administration daily for 31 consecutive days
TWx9: One administration twice weekly for 9 consecutive weeks

Exposure of rats to BN83495 and its metabolite (IDP17619) in plasma and whole blood at D132

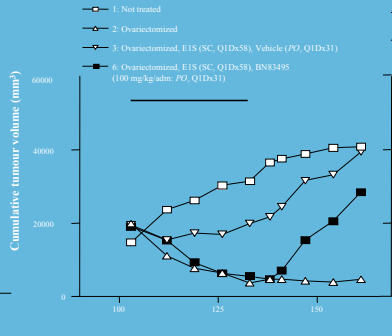
Matrix	Plasma			Whole Blood		
Groups	6	7	8	6	7	8
Treatment	BN83495	BN83495 + TAMOXIFEN	BN83495 + FULVESTRANT	BN83495	BN83495 + TAMOXIFEN	BN83495 + FULVESTRANT
BN83495						
C_{max}(ng/mL)	5433	6955	9234	7270	8031	9090
AUC(ng-h/mL)	22594	26830	35794	50401	53676	66316
IDP17619						
C_{max}(ng/mL)	1653	1660	2350	349.9	305.4	445.9
AUC(ng-h/mL)	7065	7934	9328	1858	1971	2053

Principal exposure parameters for BN83495 and IDP17619 derived from median plasma and whole blood concentrations. The BN83495 plasma exposure represent 50% of its exposure in whole blood (sequestration in red blood cells). The plasma exposure of IDP17619 represented 30% of the parent drug.

Estradiol level in rat plasma at D118

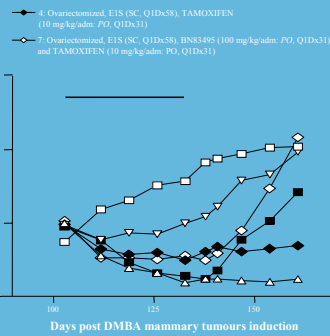


Antitumour activity of BN83495 compared to castration



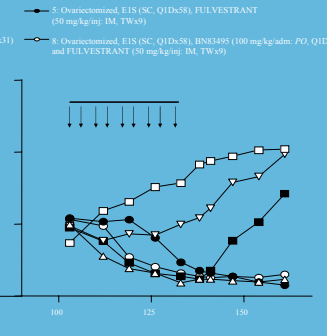
-DMBA-chemoinduced mammary tumours respond to estradiol deprivation (comparison groups 1 and 2).
-Supplementation of ovariectomized rats with E1S stimulates DMBA tumour growth (groups 1 and 3).
-Sulfatase inhibitor BN83495 inhibits significantly mammary tumour growth (groups 6 and 3).

Antitumour activity of BN83495 alone or combined with TAMOXIFEN



-The efficacy of the sulfatase inhibitor BN83495 compares well to that observed in the TAMOXIFEN treated group (groups 6 and 4).
-TAMOXIFEN inhibits significantly mammary tumour growth (groups 4 and 3).
-Combination of BN83495 with TAMOXIFEN is not deleterious to the activities of these latter (groups 4, 6 and 7).

Antitumour activity of BN83495 alone or combined with FULVESTRANT



-The efficacy of the sulfatase inhibitor BN83495 compares well to that observed in the FULVESTRANT treated group.
-FULVESTANT inhibits significantly mammary tumour growth (groups 5 and 3).
-Combination of BN83495 with FULVESTRANT is not deleterious to the activities of these latter (groups 5, 6 and 8).

CONCLUSIONS

- **The DMBA-induced mammary tumour model with ovariectomy and E1S supplementation is a suitable model to mimic postmenopausal situation in human.**
- **The new sulfatase inhibitor BN83495 caused regression of estrone sulphate-stimulated tumour growth.**
- **Oral administration of BN83495 (100mg/kg) leads to an exposure blocking the transformation of exogeneous E1S to E2.**
- **Its efficacy compared well to both inhibitors of estradiol receptors TAMOXIFEN and FULVESTRANT.**
- **Its addition is not deleterious to the activity of both ER blockers.**